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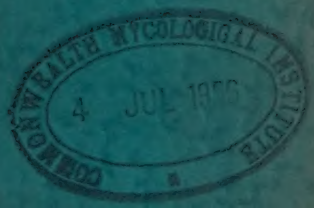

AUSTRALIAN JOURNAL OF AGRICULTURAL RESEARCH

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MELBOURNE

SPRING FROST DAMAGE TO VINES

By D. E. ANGUS*

(*Manuscript received December 7, 1955*)

Summary

Detailed temperature measurements among grape-vines, and in the inversion layer in the air overhead, are presented for two nights on which frost damage occurred. The temperatures are related to the varying degrees of damage suffered by the vines. It is concluded that the critical vine-shoot temperature was about 28½°F at the stage of development of the vines at the time of observation. The air temperature at the height of the vines was about 1°F higher.

I. INTRODUCTION

During the spring of 1955 some damage to grape vines was caused by two frosts in the Berri Irrigation District of South Australia. It is only on rare occasions that detailed temperature measurements at a number of points among the vines and in the

CORRIGENDUM

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Page 107, line 18: *For* "seed size . . . quoted by" *read*

seed, is not exceeded. However, under field conditions, where germination

the ground. The young shoots were still emerging, and ranged from a length of about 6 in. down to buds which had not burst.

Temperatures were measured with Stantel thermistors, type F2311/300 (consisting of a small bead of semi-conductor sealed in the tip of a glass tube about 3 in. long and of $\frac{5}{32}$ in. diameter). These were mounted 3 ft above the ground on wooden stakes, placed at a minimum distance of 2 ft from nearby vine rows. Sleeves of rubber tubing about 1 in. long were fitted onto the thermistors to increase their time constants for the purposes of other parts of the investigation. This also had the advantage of simulating the small vine shoots.

No radiation shields were used with the thermistors, and so they took up a slightly lower temperature than that of the air. This was accepted seeing that this holds also for the vine shoots. It is believed that the temperatures observed with the thermistors are very close to those of the actual shoots.

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Thermistors (but without rubber tubing attached) were also used to measure the air temperature at heights of 20 and 33 ft at two positions on the site. Cables from all the thermistors ran back to a central point, where a large number of temperatures could be read very rapidly. Temperatures were read at intervals throughout the two nights.

On each afternoon following a frost a careful survey of damage to the shoots was made. Damage was found to vary with the contour elevations of various sections of the planting, and also with the protection obtained at different distances from a wind machine which was under test at the time. Varying degrees of damage at different positions can therefore be correlated with the temperature records from these positions. The damage is recorded as the percentage of shoots which were killed.

III. Meteorological Conditions

The conditions during the two nights were completely different. On the night of September 17/18, although the sky was cloudless a strong drift (about 3 m.p.h. at 15 ft) continued from early evening to about 4 a.m. The temperature was therefore maintained at a comparatively high level, and at 4 a.m. was about 34°F among the vines. The air drift then stopped quite suddenly, and in the next half-hour the temperature fell about 4°F. In the ensuing $1\frac{3}{4}$ hr till sunrise the temperature fell a further 2° or 3°.

This frost was in the nature of a "black frost", no dew deposition being detected by sight or touch either on long grass or on the vine shoots. The local meteorological observing station, situated on high ground a few miles away, indicated, however, a dew-point of about 35°F on the previous afternoon and the following morning.

The damage suffered by the vine shoots was very slight, being only about 5 per cent. outside the range of the wind machine, and this damage was neglected in estimating the much heavier damage suffered the following night.

On the night of September 18/19 the sky was again cloudless, but there was practically no air movement all night. At 15 ft the drift (as indicated by the smoke from a lighted match) was never more than about $\frac{1}{3}$ m.p.h., and during some portions of the night no movement could be detected at all. Early in the night smoke from a nearby fire rose vertically to a height of about 40 ft, and then moved horizontally in a thin straight line. This showed that there was air movement at this height, but without any turbulence.

Sensitive anemometers placed among the vines at a height of 3 ft showed no air movement during most of the night. Such very calm conditions allowed a rapid fall of temperature early in the evening, and by midnight the temperature among the vines was about 31°F. Temperatures continued to fall steadily all the rest of the night, and a little before

sunrise reached a minimum of about 26½°F at the lowest contour elevation.

IV. Results

Figure 1 shows the temperatures in the inversion layers on the two nights. Two features are worth noting. On the night of September 17/18 the very rapid fall of temperature at vine level (see Fig. 2) when the drift stopped resulted in a comparatively strong inversion. Under springtime

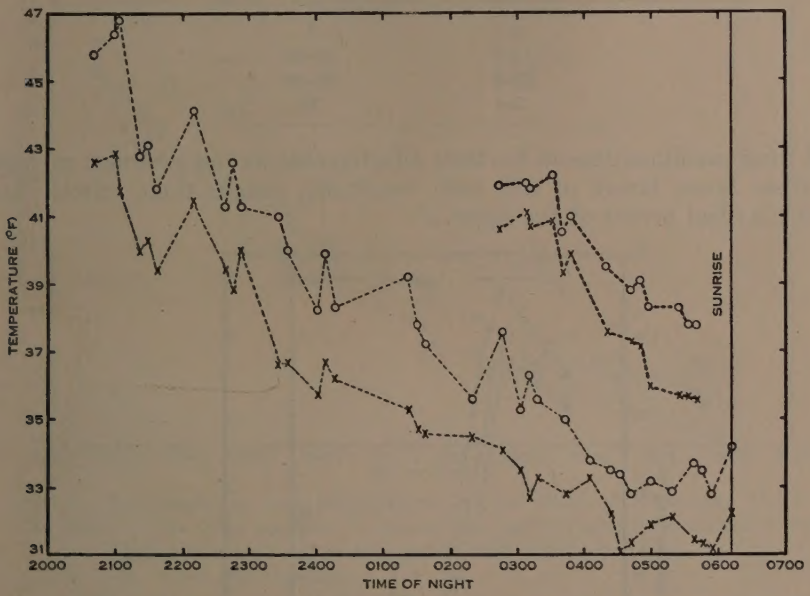

Fig. 1.—Air temperatures at 20 and 35 ft on the nights of September 17/18 and 18/19, 1955.

○ -- ○ 33 ft, September 17/18.
×---× 20 ft, September 17/18.
○---○ 33 ft, September 18/19.
×---× 20 ft, September 18/19.

conditions of generally higher temperatures, however, a sharp fall in temperature like this towards dawn is not usually severe enough to cause damage.

On the night of September 18/19 the air was stratified, owing to the very small amount of air movement. Any small vertical air currents resulted therefore in large temperature fluctuations at any given height, and this is evident in Figure 1, especially during the first half of the night. After some hours of steady conditions, the inversion strength in the lowest 30-40 ft decreased (a phenomenon which has also been observed in other places), and in the critical last few hours before dawn was somewhat weak.

It is conditions such as occurred on this night which make for damaging temperatures in spring. Since protective methods using heaters

TABLE 1
DAMAGE TO VINES AFTER VARIOUS PERIODS BELOW THE CRITICAL TEMPERATURE

Time below 28½°F (hr)	Percentage of Shoots Killed
½-1	5
1½-2	20-25
2½-3	30-40
3-4	75

and wind machines depend for their effectiveness on the inversion strength in these lower layers of air, such conditions reduce their effectiveness at the critical period of the night.

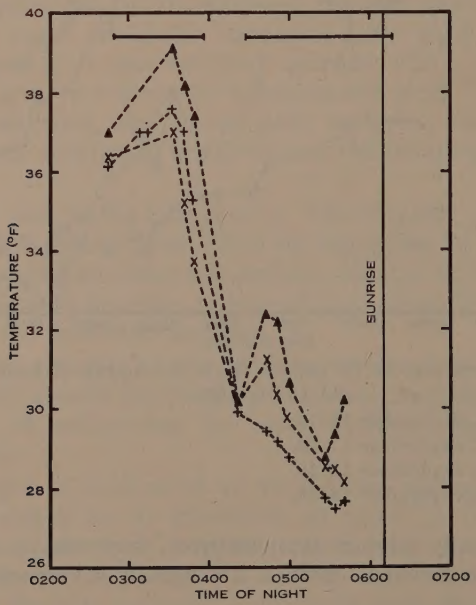

Fig. 2.—Representative vine temperatures on the night of September 17/18. The effect of the wind machine in raising temperatures when switched on can be seen.

▲----▲ No damage.
×----× 5 per cent. damage.
+----+ 5 per cent. damage.
——— Wind machine operating.

The critical temperature for the vine shoots at their stage of development at this time appears to be about 28½°F. It is stressed that this is the

actual vine temperature, which would be a degree or so below that shown by a shielded thermometer at the same height.

The temperature shown in Figure 2 as being between 28° and 28½° for a short period before dawn was observed at a position just beyond the limit of the area which was completely protected by the wind machine. These temperatures were the highest at which any damage was evident.

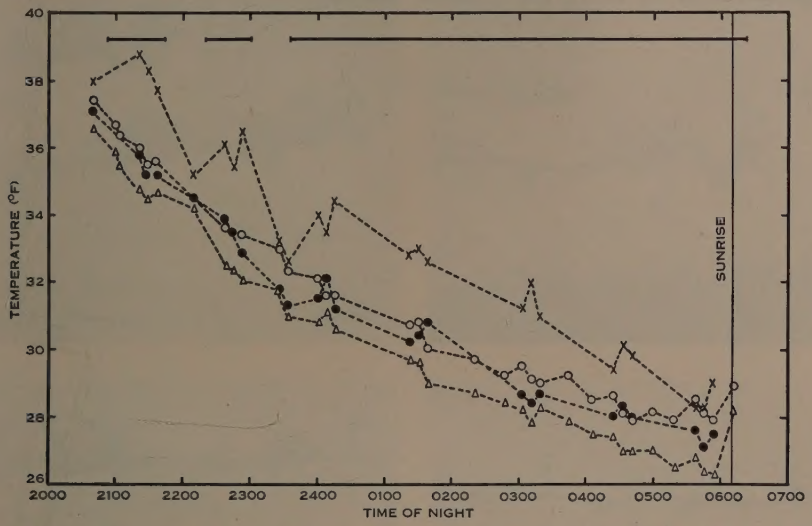

Fig. 3.—Representative vine temperatures on the night of September 18/19. The effect of the wind machine in raising the temperature when switched on can be seen at one position which was within its range.

×---× 5 per cent. damage.
○---○ 20 to 25 per cent. damage.
●---● 30 to 40 per cent. damage.
△---△ 75 per cent. damage.
——— Wind machine operating.

On both nights, wherever the temperature did not fall to 28½°F no damage occurred. The amounts of damage after different periods below this critical temperature are shown in Table 1.

Figures 2 and 3 show the fall in temperature at representative positions, for different amounts of damage. A similar pattern of damage at the various temperature levels was obtained at more than a dozen positions. In these figures the effect of the wind machine in raising temperatures when switched on can be seen for those positions within its range.

Plate 1 shows the varying degrees of damage to the vine shoots. Fortunately the frosts occurred before bud burst was complete, and the dormant primary buds remained practically undamaged. A large number of fertile shoots therefore emerged after the frosts.

An estimate of the crop yield was made about 6 weeks later by the grower. In the area where the damage did not exceed 25 per cent., increased bunch and berry size and probably better setting and sugar content, in addition to the further fruitful bud burst already mentioned, ensured that a normal harvest could be expected. In the area where the damage was severe, these factors appeared likely to give a harvest expectation of 60 to 70 per cent. of normal.

SPRING FROST DAMAGE TO VINES

Fig. 1.—Healthy shoots showing no damage.
Fig. 2.—Shoots in the area of 10 per cent. damage (shrivelled black shoots are dead).
Fig. 3.—Shoots in the area of 35 per cent. damage.
Fig. 4.—Shoots in the area of 75 per cent. damage.

NITROGEN STUDIES ON BLACK SOILS FROM THE DARLING DOWNS, QUEENSLAND

I. SEASONAL VARIATIONS IN MOISTURE AND MINERAL NITROGEN FRACTIONS

By A. E. MARTIN* and J. E. COX†

(*Manuscript received January* 23, 1956)

Summary

A study of the equilibrium levels of moisture, nitrate nitrogen, and ammonium nitrogen is reported on two representative black soils (developed from basaltic colluvium and alluvium respectively) from the Darling Downs for the years 1952-1954 inclusive. Both soils were under native grassland in 1952; a portion of the sampling area at each site was cultivated early in 1953 and left fallow for the rest of the sampling programme.

The moisture contents of surface (0-6 in.) grassland and fallow plots varied directly with rainfall. Subsoil under grass showed low moisture contents which increased only after heavy rain but decreased thereafter, probably owing to transpiration. Under fallow, subsoil moisture was higher and showed significant fluctuations, suggesting that losses occurred by slow liquid flow or vapour movement at moisture contents between field capacity and the wilting point.

Ammonia-nitrogen contents at the surface were lower than for comparable soils in temperate regions and showed a significant decrease with depth; values for the corresponding fallow plots were similar in amount and varied similarly with depth. Surface soils showed minor fluctuations with season, being higher in summer. Under incubation conditions at 25°C, ammonia nitrogen rarely fell below 1.0 mg/kg oven-dry soil, which suggests that this is the minimum threshold concentration of ammonia nitrogen in these soils for the nitrification process.

Nitrate nitrogen showed a significant rise in summer on the colluvial basaltic soil under grass. The soil developed on basaltic alluvium showed no such seasonal trend and contained uniformly low amounts of this fraction. Cultivation resulted in a sharp rise in nitrate nitrogen in both soils, the wetter alluvial soil showing a longer time lag. There was some evidence of nitrification in the 6-12 in. depth at one site, but below this rises in nitrate nitrogen, which were correlated with rainfall and increases in moisture content, could be accounted for by leaching. Incubation tests gave rise to highly variable nitrate nitrogen contents and such tests were found unreliable in these soils, probably owing to imperfect aeration of the laboratory sample.

Analyses for total nitrogen of several alluvial black soils from the same area showed a progressive decline in this constituent with increasing periods of cultivation; in the first 25 years of cultivation about 0.8 per cent. of the total nitrogen is lost per annum. This value agrees with the nitrate nitrogen accession in fallow subsoil, suggesting that leaching of nitrate formed at the surface is a major factor determining losses in total nitrogen. The implications of these findings are discussed.

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I. Introduction

A large proportion of the soils on the Darling Downs are deep black soils of fine texture, developed on basalt or basaltic alluvium and devoted mainly to the growth of cereal crops and to dairying. The use of nitrogenous fertilizers to increase cereal productivity is not a normal agricultural practice on these soils, probably for economic reasons. Waring (1952) in a comparison of two sites in this region with, respectively, 20 and 5 years' continuous cultivation, found no indication of a decline in fertility. On the other hand, Andrew, Kipps, and Barford (1952), in pot experiments using herbage species, indicated responses to both nitrogen and sulphur on soils near the Condamine River. Furthermore, as many of these soils are well supplied with exchangeable potassium and give rise to generally high values for phosphorus soluble in dilute sulphuric acid, some degree of response to added nitrogen may not be unexpected. It appeared necessary, therefore, to undertake a study of the mineral nitrogen equilibria in these soils. Results presented in this paper show seasonal changes in nitrate nitrogen, ammonia nitrogen, and moisture content, under grass and fallow, for two typical soils during the years 1952-1954 inclusive. As a corollary to this work, further studies were made on some aspects of the nitrifying process in these soils; the results of these experiments are described in paper II of this series.

II. Experimental

(*a*) *Sampling Sites*

Three sites, all in native grassland, were originally selected for sampling. Two (sites A and B) occur on low basaltic rises in the Mt. Gowrie area, about 15 miles west of the Great Dividing Range at Toowoomba. These soils are colluvial or residual black soils on basalt and are classed by Thompson and Beckmann (unpublished data) as Wilton clay (site A) and Irving clay (site B). The third site (site C) is located in the Mt. Tyson area about 30 miles west of Toowoomba; the soil is a deep black earth, showing gilgai microrelief, and developed from basaltic alluvium; it is described by Thompson and Beckmann (loc. cit.) as Waco clay. At site B the 0-6 in. horizon has a total nitrogen content of 0.21 per cent. with a pH (1: 5 suspension) of 7.3; at site C the surface layer shows respective values of 0.17 per cent. and 8.4.

The climate is temperate subhumid, with an average annual rainfall of 25 in., of which about two-thirds falls in the period December-April. The mean annual temperature is approximately 65°F and frosts occur occasionally in winter, particularly on the alluvial plains.

At each site the vegetation is dominated by native grasses and includes tall oat grass (*Themeda avenaceae* (F. Muell.) Durand & Jackson), Queensland blue grass (*Dichanthium sericeum* (R. Br.) A. Camus), spear grass (*Stipa* spp.), and *Danthonia* spp. At sites A and B, growth of burr medic

(*Medicago denticulata* Willd.) occurs during late winter and early spring; this species was absent at site C.

(*b*) *Sampling Technique*

At each site, an area 1 chain square was fenced off and a plot 18 by 15 yd marked out within. Each plot was divided into 30 randomly numbered subplots, each 3 yd square, from which the soil cores were taken.

During the first year (1952), when all sites were under grass, each plot was sampled at 4-weekly intervals. One core of the 0-6 in. surface soil was taken from each subplot with a 4-in. Iwan auger and each group of five numerically consecutive cores was bulked. The bulk samples were freed from roots and broken down by hand (or with a pestle and mortar during dry periods) to approximately $\frac{1}{4}$-in. aggregates. One-hundred–gram samples were weighed into 2-lb shaking bottles and each treated with 500 ml of extractant. The bottle lids were firmly screwed on after effervescence from soil carbonate had ceased. Further 100-g samples were transferred to similar bottles, firmly sealed without addition of extractant, and reserved for the incubation test. Finally a small portion (25-50 g) of each bulk sample was transferred to an air-tight tin for moisture determination. At each site six replicates for mineral nitrogen, moisture, and incubation studies were thus collected at each sampling.

Half the sampling area at both sites B and C was cultivated with a rotary hoe on January 20, 1953, and maintained free of grass and weeds until the end of the sampling programme. The sampling technique was modified during 1953 and 1954 in order to follow the movement of mineral nitrogen and moisture into the subsoil. By using random coordinates four cores were taken, to a depth of 24 in., on each of the grass and fallow plots. Each core was divided at 0-6, 6-12, and 12-24 in. depths, thus giving 12 samples from each treatment for analysis. For 1953-1954, samples for incubation were restricted to site B. Site C was sampled at 4-weekly intervals and site B at 8-weekly intervals for the rest of the programme.

All weighing operations and additions of extractant were performed in the field, the maximum time interval between the sampling of a soil and treatment of it with extractant being 5 min. Changes in mineral nitrogen fractions which occur on disturbance of the soil were thus minimized.

In spite of the modification in sampling procedure introduced following ploughing, variation within treatments was generally low and enabled some comparisons to be made over the 3-year period. Since the fallow treatments were unreplicated it was not possible to formulate a satisfactory analysis of variance and obtain values for interaction. This would have involved a more ambitious programme of sampling and analysis than was at that time possible. It was therefore only possible to compare depths and sampling dates within treatments.

(c) *Methods of Analysis*

At the laboratory, all soil suspensions were shaken mechanically for 1 hr, allowed to stand overnight, and filtered under suction through a Buchner funnel containing a Whatman No. 42 paper. In the initial trials some fungal contamination was apparent in spite of the presence of free copper ion and thereafter a few drops of chloroform were added to each filtrate. Toluene was found to interfere markedly with the determination of nitrate, owing to its preferential nitration.

(i) *Extracting Solution.*—This solution consisted of N sodium sulphate which was 0.2N with respect to sulphuric acid. Copper sulphate was added to give a final concentration of 0.02N, to reduce microbial changes.

(ii) *Determination of Ammonia and Nitrate.*—Ammonia nitrogen was determined by distillation of an aliquot of the soil extract with freshly ignited magnesium oxide in an all-glass apparatus, followed by nesslerization of the distillate using an Ilford 601 filter for photometric comparisons. The Nessler solution used was stronger than that recommended by Vanselow (1940), but the concentration of the reagent in the final solution was approximately the same as that used by this worker. Nitrate was determined using 3,4-xylenol; the nitroxylenol formed was distilled in an all-glass apparatus before developing the colour with alkali. These methods have been developed by Piper and Lewis (unpublished data) and were found highly satisfactory.

(iii) *Moisture.*—Samples of fresh soil (20-30 g) were dried for 18 hr at 105°C and the loss in weight recorded as moisture.

(iv) *Incubation Tests.*—During 1952, three 100-g portions, one from each of three bulk samples from sites B and C, were brought to field capacity by addition of distilled water. Another three portions, one from each of the three remaining bulk samples, were treated similarly but the added water contained ammonium sulphate corresponding to an application of 3 cwt/acre (3.18 g ammonia nitrogen per 100 g oven-dry soil). The incubation bottles were then weighed, placed in the dark in a constant temperature room at 25°C, and any losses in weight made up with ammonia-free water every 2-3 days. After 5 weeks, 500 ml of extractant were added to the contents of each bottle and the filtered extract analysed for ammonia nitrogen and nitrate nitrogen as before. Surface samples from grass and fallow at site B only were collected for incubation during 1953-1954; no additions of ammonia nitrogen were made in this series.

III. Results

Results for all mineral nitrogen fractions and moisture were calculated on an oven-dry basis: allowances were made both for the weight of water in the fresh samples and for the diluting effect of soil moisture on the extractant. Sampling at site A was discontinued after 1952 as most

results closely resemble those from site B; data from site A are not therefore included in this paper.

(*a*) *Moisture*

Figures 1 and 2 show the seasonal variation in moisture content at sites B and C respectively. Values for the 6-12 in. depth were omitted

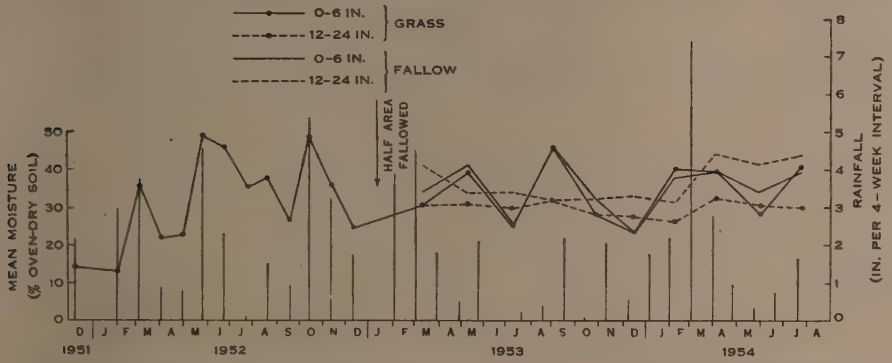

Fig. 1.—Seasonal values for moisture and rainfall at site B. Curves represent moisture fluctuations and vertical lines rainfall per 4-week period. Mean standard deviations ($P = 0.05$) are: *Grass*, 1952 — 1.70 for time means; 1953-1954 — 2.42 for time means, 0.77 for depth means. *Fallow*, 1953-1954 — 2.31 for time means, 0.70 for depth means.

from the diagrams for the sake of clarity, as at this depth moisture contents were generally intermediate in magnitude between those at the

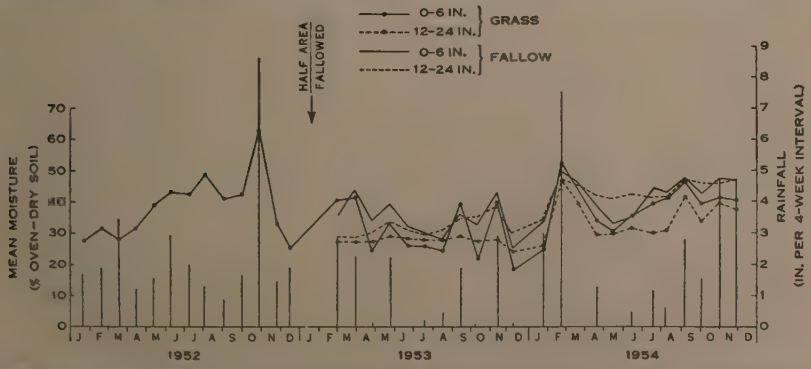

Fig. 2.—Seasonal values for moisture and rainfall at site C. Curves represent moisture fluctuations and vertical lines rainfall per 4-week period. Mean standard deviations ($P = 0.05$) are: *Grass*, 1952 — 2.04 for time means; 1953-1954 — 2.45 for time means, 0.41 for depth means. *Fallow*, 1953-1954 — 2.53 for time means, 0.47 for depth means.

other depths sampled. A general picture of the rainfall conditions is included by plotting the total rainfall recorded at each site during the

interval between samplings. For site B, 4-weekly rainfall totals are recorded in 1953-1954 when samplings were 8 weeks apart. This method of presentation is somewhat insensitive but is no more so than is justified

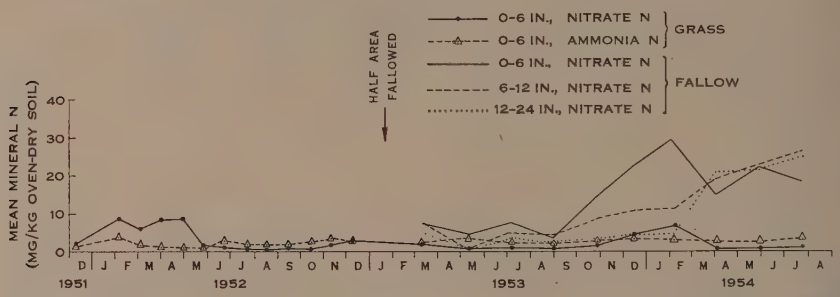

Fig. 3.—Seasonal values of mineral nitrogen under grass and fallow at site B. Mean standard deviations ($P = 0.05$) are: *Ammonia nitrogen,* 1952 — 0.4 for time means; 1953-1954 (grass) — 0.5 for time means, 0.13 for depth means; 1953-1954 (fallow) — 0.4 for time means, 0.17 for depth means. *Nitrate nitrogen,* 1952 — 1.2 for time means; 1953-1954 (grass) — 0.8 for time means, 0.3 for depth means; 1953-1954 (fallow) — 3.8 for time means, 1.1 for depth means.

by the frequency of sampling, since the purpose was only to indicate broad trends in the constituents studied rather than to attempt to establish their detailed relation to weather.

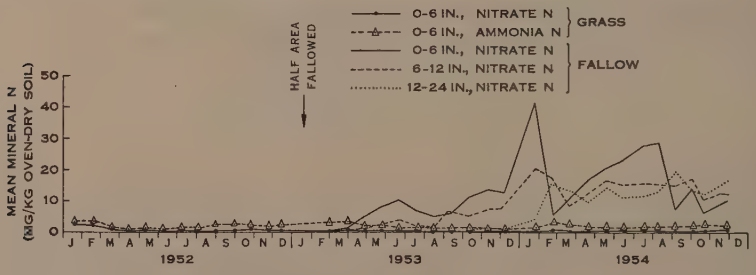
0-6 IN., NITRATE N
0-6 IN., AMMONIA N
GRASS
0-6 IN., NITRATE N
6-12 IN., NITRATE N
12-24 IN., NITRATE N
FALLOW
HALF AREA FALLOWED
MEAN MINERAL N (MG/KG OVEN-DRY SOIL)
0
10
20
30
40
50
J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D
1952
1953
1954

Fig. 4.—Seasonal values of mineral nitrogen under grass and fallow at site C. Mean standard deviations ($P = 0.05$) are: *Ammonia nitrogen,* 1952 — 0.3 for time means; 1953-1954 — 0.4 for time means (grass and fallow), 0.1 for depth means. *Nitrate nitrogen,* 1952 — 0.3 for time means; 1953-1954 — 3.8 for time means, 0.7 for depth means (fallow only).

At site B, moisture was highly variable at the surface. There were no marked seasonal effects, except that high moisture levels were sustained longer during the wet winter of 1952 than in the comparatively dry winter of 1953. There was little difference between grass and fallow samples. In subsoil layers more marked differences appeared and, in agreement with general experience, fallow subsoils were appreciably wetter. In both grass and fallow plots, the differences between depth means were highly signifi-

cant ($P < 0.01$) throughout the experimental period except in October 1953. Grassland subsoil at this site showed remarkable constancy in moisture content and appeared to be affected only by concentrated rainfall.

Variation was, again, appreciable in surface samples at site C and on the whole these differences were highly significant, but they seem to vary seasonally more than surface samples from site B; generally a higher moisture content in summer was observed, and surface moisture appeared more sensitive to rain in winter. Change to fallow conditions had a marked effect on moisture content at this site. Moisture relationships in the surface horizon of the fallow plot closely resembled those of the grass plot but

TABLE 1
MEAN VALUES FOR AMMONIA NITROGEN FOR 1953-1954
Results as mg/kg oven-dry soil

Site	Depth (in.)	Ammonia Nitrogen (mg/kg) on	
		Grassland	Fallow
B	0-6	2.2	2.3
	6-12	1.4	1.1
	12-24	0.9	0.6
C	0-6	2.0	2.0
	6-12	1.3	1.3
	12-24	0.7	0.6

were almost uniformly higher; this effect is apparent very shortly after cultivation. Moisture in the grassland subsoil remained very constant during 1953 but was affected markedly by heavy rains in February 1954. In the winter of 1954, however, the moisture level soon dropped nearly to its original value. In contrast, the fallow subsoil moisture rose continuously and significantly (except for a fall during the dry December of 1953) throughout the rest of the sampling period and at times exceeded, at a high level of significance, the moisture content of the fallow surface.

(*b*) *Ammonia Nitrogen*

Variations in ammonia nitrogen and nitrate nitrogen are plotted for both sites in Figures 3 and 4. To avoid confusion, ammonia nitrogen values for surface samples of grassland soil only are plotted in each figure, since values for surface fallow are very similar. Table 1 lists the mean values of this fraction for all depths at each site for 1953-1954. There was a significant decrease in depth with each treatment; differences between sites were very small.

Ammonia nitrogen showed a small periodicity at both sites. For all grassland surface samples there was a significant minimum value from about May to August; in the first year, fallow plots showed a similar trend but the changes were non-significant. Subsoil samples tended to follow

surface samples in seasonal changes, although at the 12-24 in. depth most changes were slight and non-significant. There was no significant change in ammonia nitrogen with time, with either fallow plot, to the end of sampling.

(*c*) *Nitrate Nitrogen*

Table 2 shows mean values of this fraction for both sites at each depth. Within treatments, nitrate nitrogen at all depths decreased significantly in magnitude except for grassland at site C, where it was already extremely low in the surface horizon. Nitrate nitrogen was significantly higher at the start of sampling in 1952 than in the corresponding period in 1953. This may have arisen through inadvertent sampling of relatively fresh patches of excreta soon after enclosure of the plots, although this was at all times consciously avoided as far as possible.

TABLE 2
MEAN VALUES FOR NITRATE NITROGEN FOR 1953-1954
Results as mg/kg oven-dry soil

Site	Depth (in.)	Nitrate Nitrogen (mg/kg) on	
		Grassland	Fallow
B	0-6	1.6	14.2
	6-12	0.5	11.3
	12-24	0.1	8.8
C	0-6	0.3	12.7
	6-12	0.2	9.4
	12-24	0.2	7.0

At site B, significant seasonal trends occurred for surface soils under grass. Nitrate nitrogen built up appreciably during the two summer periods covered, while in winter very low values were recorded. The 6-12 in. layer under grassland also showed a significantly higher nitrate nitrogen in the period December 1953-February 1954, although this rise was not sustained. The deepest grassland layer showed no significant change with time and the nitrate nitrogen was always extremely low. Fallow soils at this site showed marked changes following cultivation; sampling occurred about 7 weeks after ploughing and an immediate significant rise in nitrate nitrogen was recorded. After some fluctuations the level fell significantly during August 1953 at the end of a dry winter. Following opening rains in September and with rising temperature, nitrate rose steadily in the surface to a maximum of nearly 30 mg nitrogen/kg in February 1954. Very heavy rain at the beginning of March caused a drop in the surface layer to 15 mg/kg; an immediate rise in nitrate nitrogen was recorded in both subsoil layers. This was particularly apparent in the deepest layer, which had suffered only minor fluctuations over the previous 12 months. Peak values for nitrate nitrogen coincided with a smaller peak in the grassland soil. The 6-12 in. layer showed a

fairly uniform rise over the experimental period and was still rising when sampling terminated.

At site C, except for initial values for nitrate nitrogen of about 2 mg/kg, caused probably by previous contamination with urine, grassland samples showed very low values at all depths for the 3-year period. The maximum value recorded in the surface layer was 0.9 mg/kg. Subsoil layers showed progressively lower nitrate contents and for long periods, especially in winter, the amount of nitrate present in the soil extracts was equal to that in the blank solutions. Fallow plots were sampled about 3 weeks after cultivation and nitrate nitrogen values had not at this stage shown any change from those in grassland soils. It is noteworthy that January and early February in 1953 were unusually dry and the moisture contents of the soils were low. Some heavy rain in mid February caused a significant rise of nitrate nitrogen in the fallow surface, and, except during the dry June-September period of 1953 when nitrate nitrogen fell significantly, the general level rose to very high values in January 1954. Subsequently, during the relatively mild and rather moist winter of 1954, surface nitrate values rose steadily until the opening rains in September-November coincided with a further fall in nitrate levels. Subsurface horizons showed a considerable lag in nitrate accumulation. The 6-12 in. depth, apart from a significant rise to 4 mg/kg in June 1953, did not become markedly richer in nitrate until the period from September to December, when levels paralleled those at the surface but did not approach them in magnitude. The deepest horizon showed no significant rise until January 1954, and following heavy rain in February and March 1954, values in this layer rose to levels comparable to those in the 6-12 in. depth. Towards the end of 1954 the deepest layer under fallow showed significantly higher nitrate contents than either the surface or subsurface layers.

(*d*) *Incubation Studies*

The object of including incubation studies was, following Richardson (1938), to isolate the "down-grade" portion of the nitrogen cycle and endeavour to observe fluctuations in readily decomposable organic matter. Figure 5 summarizes the results, in terms of mean net nitrate production (i.e. total nitrate after incubation less field nitrate) at each sampling date. During 1952 there was clearly no seasonal correlation, while agreement between replicates was abnormally poor. It was realized during 1952 that soil moisture content had an inverse relation to net nitrate production. Examination of results for both sites showed a highly significant inverse correlation between the net production of nitrate nitrogen and the moisture content at sampling (see Table 3). This does not necessarily imply a causal relation between the two populations and in any event this correlation was non-significant at site B in 1953-1954 under either grass or fallow. It is of interest in this connexion that 1952 was an abnormally

wet year, whereas 1953 was very dry. Clearly, a high soil moisture content is conducive to puddling during sampling, leading to greatly reduced opportunities for adequate oxygen diffusion into the aggregates. In view of the almost constant value of ammonia nitrogen found after incubation, discussed later, we must assume that nitrification is complete but that the nitrate produced is subjected thereafter to partial denitrification. The final value of nitrate nitrogen found would then be the resultant of these

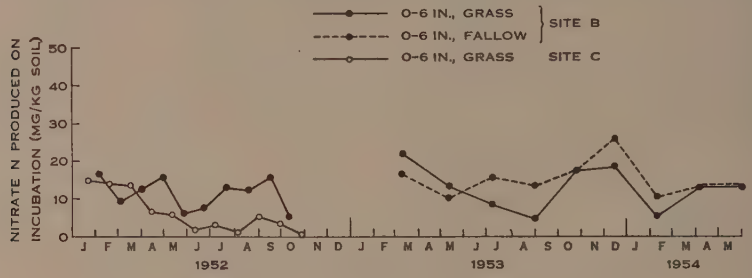

Fig. 5.—Soil nitrate production on incubation at 25°C.

two processes in soils of high water content. The recovery, as nitrate nitrogen, of ammonia nitrogen added to the 1952 samples averaged 85 per cent. for site B but only 76 per cent. for site C, a much wetter soil in general. Soil from site C at the lower plastic limit showed a moisture content of 43 per cent.; below this level puddling would be unlikely but aeration within the aggregates would still be of a low order. Assuming a bulk density of 1.13 g/c.c. for surface soil at site C, then, at a moisture content of 40 per cent., 15 per cent. of the total pore space would be

TABLE 3

CORRELATION OF FIELD MOISTURE AND NET NITRATE PRODUCED ON INCUBATION AT 25°C

Treatment		Site B	Site C
1952	Grassland	$r = -0.92^{***}$	$r = -0.87^{***}$
1953-1954	Grassland	$r = -0.47$ N.S.	—
	Fallow	$r = -0.52$ N.S.	—

occupied by air. At 35 per cent. moisture, the air fraction would rise to 20 per cent., so that during a considerable part of the year most aggregates would be poorly aerated even if held at their field moisture content, while addition of water to field capacity would still further decrease aeration. For incubation tests to have their proper meaning on soils of this type some technique of rapid drying to a standard moisture content must be developed.

Almost without exception, the ammonia nitrogen found on incubation was less than that found in the field samples; the mean values for field

and incubated samples are given in Table 4. Similar results are given by Barnes (1950) for a Woburn soil although his ammonia nitrogen values are higher. Field values are all significantly greater than those found after incubation; individual incubated samples show very constant values and remain unaffected either by addition of ammonium ion prior to incubation or to season. It seems likely therefore that a mean value of about 1 mg ammonia nitrogen/kg oven-dry soil represents the threshold level in these surface soils below which nitrification is greatly retarded or stopped. Individual values of field ammonia nitrogen of less than 1 mg/kg were recorded on only four occasions at the two sites, and these were all during April-June 1952.

TABLE 4

MEAN VALUES OF AMMONIA NITROGEN BEFORE AND AFTER INCUBATION AT 25°C

Results as mg/kg oven-dry soil

Site	Treatment		Field Ammonia Nitrogen (mg/kg)	Incubated Ammonia Nitrogen (mg/kg)	
				No Added Nitrogen	Added Nitrogen
B	1952-1954	Grass	1.94	1.07	1.07
	1953-1954	Fallow	2.30	1.01	—
C	1952	Grass	1.82	1.10	1.10

IV. DISCUSSION

(*a*) *Moisture*

The most interesting features of the moisture levels of these soils concern the relation between surface and subsoil moisture contents. While moisture in the 0-6 in. layer, in fallow and grass, is clearly sensitive to weather, accessions of water at 12-24 in. under grass are brought about only by concentrated rainfall. The protective effect of surface vegetation in this connexion is clearly seen in comparing subsoil moisture for the two treatments at both sites in Figures 1 and 2. It is less easy to assign the cause for decreasing subsoil moisture after the wet season. Since all samples were below field capacity during late February-May 1954 and since the rainfall data indicate that wetting to values greater than this was unlikely at that depth, losses in moisture must be due to vapour movement or transpiration. The fact that grassland subsoil dries out to a much lower level than fallow during this period suggests that transpiration is a significant factor in controlling water relations at this depth. Waring (1952) found also that moisture losses on a similar alluvial black soil were higher under wheat than under fallow, especially in the 1-3 ft zone. However, the results reported here show a significant decline in fallow subsoil moisture over this period which cannot be accounted for by rapid

drainage or transpiration. Evaporation from the surface seems unlikely since loss by this means is notoriously slow (Penman and Schofield 1941), so that moisture changes at depth must be accounted for by vapour transfer and slow liquid flow.

Determinations of 15-atm moisture content by the pressure membrane apparatus gave values of 38 per cent. (oven-dry basis) on the surface and 12-24 in. depths at site C. Samples from site B were not examined in this way but their 15-atm values would approximate to 30 per cent., judged on the relative clay contents. It is seen from Figures 1 and 2 that for the whole of 1953, moisture in grassland subsoil was probably at or well below "wilting point". Apart from moisture accessions already noted during heavy rain it is surprising that these subsoils at both sites remain abnormally dry for such long periods. It is, however, possible that although a bulk sample may show low values there may be channels of soil at much higher levels of moisture which are able to support isolated roots. The growth of grass at each site is correlated with surface moisture during the growing season (November-April) but seems quite independent of subsoil moisture. During spring and early summer isolated showers will result in regrowth which dies back during subsequent dry spells; these showers are not intense enough to affect subsoil moisture levels.

(*b*) *Ammonia Nitrogen*

The mean values for ammonia nitrogen quoted in Table 1 seem to be independent of soil type and are much lower than those reported for British permanent grassland soils (Eggleton 1934; Richardson 1938). There is no apparent drop in this fraction on cultivation in the subsequent 2 years; equilibrium levels in both grass and fallow are low, and in the surface layer generally approach the limiting threshold ammonia content of about 1 mg kg noted previously. Ap Griffith (1951) also noted ammonia nitrogen levels of about this order in Uganda soils. This implies that nitrification has a greater depleting effect on ammonium ion than in cooler climates. The seasonal drop in this fraction during the colder and drier months cannot be explained from the results although the fluctuations are small.

Ammonia nitrogen values given by Waring (1951) for that season on similar soils are much higher at depth; the mean values for three sites (June-October) are 1.9, 3.3, 3.5, and 1.8 mg ammonia nitrogen kg oven-dry soil at the 0-3, 3-12, 12-24, and 24-48 in. depths respectively. These soils had been in cultivation for 5-20 years at the time of sampling. It is difficult to account for the discrepancy between his results and our own, which show a significant decrease with depth down to 2 ft.

(*c*) *Nitrate Nitrogen*

Although surface nitrate values under grass at both sites cannot be compared statistically for the whole period, there are notable differences

which may be suggestive. The extremely low values at site C are independent of season and are sustained at this low level throughout the sampling period. Site B, however, shows significant summer maxima and generally higher nitrate levels which may indicate more favourable conditions for nitrification in the more open colluvial black soils. The growth of burr medic is common on this site during winter, and this may favour the process; further, the site C soil is the more alkaline by 1.1 pH units which, as will be discussed more fully in paper II of this series, may adversely affect the nitrifying potential of this soil.

At site B under fallow, there was no marked rise in nitrate in the 6-12 in. zone until October 1953. Since rainfall during this period was confined to showers of (on average) 0.24 in. per wet day, leaching of nitrate from the surface would be negligible. We can conclude therefore that some nitrification is taking place in this layer although, compared with the surface, to a greatly reduced extent. A study of the nitrate levels in the deepest layer leads us to suspect that there is here virtually no nitrification, even though moisture and temperature conditions may be favourable. This is expected in view of the very low ammonia levels in this zone.

In the fallow soil at site C the first rise in surface nitrate following cultivation showed a longer lag period before rising to moderate values in June 1953, further confirming the lower nitrifying potential of this soil. As with site B, the rise in the 6-12 in. zone can be partly attributed to nitrification *in situ* and partly to accessions from leaching. The deepest layer accumulates nitrate presumably only by leaching from the surface. High nitrate accumulation has been noted in other tropical soils (Wells 1937; Jewitt 1950).

(*d*) *Losses in Nitrogen under Cultivation*

Short or long fallowing is common on the Darling Downs as a means of conserving soil moisture for cereal crops. While this practice has the desired effect, our results show that it is accompanied by losses of nitrate from the 0-6 in. layer. Eventually, continued fallowing and cropping in the absence of a balanced rotation may lead to measurable losses in soil nitrogen and, presumably, also in organic matter. Surface samples (0-6 in.) of Waco clay, representing various periods of cultivation for wheat growing, were collected from a number of properties on the Darling Downs and analysed for total nitrogen. At each property, samples of virgin grassland soil were also taken for analysis from as near as possible to the arable sample. Most representatives of this soil series show some degree of gilgai microrelief so that samples were taken from puff and depression phases. Normally the depression phase contains more total nitrogen than the puff and this effect tends to persist with cultivation, though to a lesser extent. Mean values of total nitrogen for puff and depression were therefore calculated. The results are illustrated in Figure

6, which shows the dependence of loss in total nitrogen on the time of cultivation. Loss in nitrogen was calculated as a percentage for each property; variation between total nitrogen on virgin soils from different properties precluded the use of a mean value for the uncultivated soils. Two values are questionable and were obtained from soils known to be heavily silted during recent flooding; they showed about 40 per cent. loss in nitrogen after only 8-14 years of cultivation and are not, therefore, included in Figure 6.

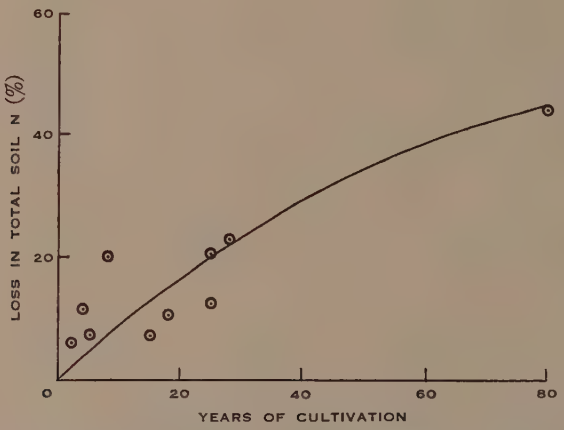

Fig. 6.—Relation between total soil nitrogen and time of cultivation on Waco clay.

The freehand curve drawn through the points shows an average loss in nitrogen of about 0.8 per cent. per annum over the first 25 years. Assuming an average total nitrogen value of 0.16 per cent. for these soils, and that the surface 6 in. of this soil weighs 1.5×10^6 lb/acre, this represents an annual loss of 19 mg nitrogen/kg oven-dry soil. For the 12-24 in. layer at site C the mean content of nitrate nitrogen is 13 mg/kg, which can be considered the total loss of nitrogen from the surface layer by leaching. In view of the errors involved in this type of calculation the results are surprisingly concordant and suggest that nitrogen losses from the surface layer by leaching are a major factor in the nitrogen economy of these soils when used for wheat growing.

Shutt (1910) estimates losses of nearly one-third of the total nitrogen in the surface 8 in. of some Canadian prairie soils after nearly 25 years of cultivation. In a study of the nutrient status of soils in wheat-growing areas in New South Wales Hallsworth, Gibbons, and Lemerle (1954) estimate a 16-26 per cent. loss in total nitrogen on chernozemic soils that had grown 10 or more wheat crops. This approximates to a 2 per cent. loss of total nitrogen per annum, a more serious depletion of nitrogen reserves than is indicated by our results. There is fragmentary evidence

of positive responses on the Darling Downs to nitrogen fertilizers for some herbage species (Andrew, Kipps, and Barford 1952) and to millet and oats (Bisset and Andrew 1953), but on the other hand there are no reliable suggestions at present of a marked decline in wheat yield or in the nitrogen content of grain. The results reported here under fallow would not suggest nitrogen deficiency levels. It is problematical, however, how long the fallow-wheat "rotation" can be maintained without serious losses of nitrogen and of organic matter. Some form of compromise must be reached to preserve the advantages of fallowing with regard to moisture levels but remove the disadvantages of losses in nitrogen and organic matter which may lead to structural deterioration.

V. Acknowledgments

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NITROGEN STUDIES ON BLACK SOILS FROM THE DARLING DOWNS, QUEENSLAND

II. THE NITRIFYING ACTIVITY OF SUBSURFACE HORIZONS

By A. E. Martin* and J. E. Cox†

(*Manuscript received January* 23, 1956)

Summary

Rates of nitrification of a black clay soil were studied with the percolation technique, using samples from various depths down to 3 ft. There was a marked decrease in nitrification rate with increasing depth, the successive curves showing longer lag periods. Deep samples, which contain more chloride and have a higher pH value than those from the surface, accumulated large quantities of nitrite. Chloride, added to the percolators in concentrations equal to those found in the profile, had no marked effect on nitrite accumulation but slightly reduced the rate of nitrate production. The percolation of surface samples with ammonium sulphate solutions with the pH increased by alkali additions resulted in marked nitrite accumulation comparable with that found in deeper layers in presence of ammonium sulphate only. Continued repercolation at high pH values gave evidence of adaptation of the soil organisms to alkaline conditions; the percolate pH at which maximal nitrite accumulation was recorded rose with successive treatments.

It was concluded that the decrease in nitrifying capacity with depth is related to reduction in numbers of active organisms and to the partially selective effect of high pH on the oxidation of nitrite.

I. Introduction

Data presented in paper I of this series (Martin and Cox 1956) showed that, in black soils from the Darling Downs, nitrate is found in subsurface layers of fallow soils. Waring (1952) similarly found high amounts of this fraction at depths of 2-4 ft. It was suggested from these results that this nitrate accession was the result of leaching and, further, that active nitrification did not take place in the 1-2 ft layer. In order to test this hypothesis, and to study further some aspects of the nitrifying process in these soils, rates of nitrification of dilute ammonium sulphate solutions, using soil samples from various depths, were examined with the percolation technique.

II. Experimental

(*a*) *Preparation of Samples*

The sampling site was located within the experimental area near Mt. Tyson (site C, Waco clay) used in the field studies reported in the

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preceding paper. This soil is a black alkaline heavy clay developed on basaltic alluvium and has a wide distribution on the Darling Downs. Soil samples were obtained from a pit at the following depths: 0-3, 3-6, 6-12, 18-24, and 30-36 in. Care was taken to avoid contamination of each sample with material from other layers. All samples were air-dried and ground; the 1-4 mm fraction was sieved out and stored in air-tight tins for the percolation tests.

(*b*) *Percolation*

Percolation units were similar to those described by Lees (1947). Twenty grams of the 1-4 mm samples was weighed and a 3-5 g portion added to the soil column (containing a glass wool plug) followed by another loose plug of glass wool. Further small portions of the weighed sample were added in the same manner and separated with glass wool. In this way, free, and as far as possible, fully aerobic percolation was ensured throughout the experiment. About 1 g calcium carbonate was added to the top of the soil in each column to prevent the development of low pH values during nitrification; each soil was then percolated with 120 ml 0.01N ammonium sulphate, and percolation tests at each depth were made in duplicate. One-ml samples of percolate were removed at intervals for the determination of nitrite and nitrate. All units were housed in a constant temperature room at 25°C (± 0.5°C) throughout the experiment; losses by evaporation were estimated by weighing and made up with ammonia-free distilled water. No additional allowances were made for withdrawals for analysis.

(*c*) *Methods of Analysis*

(i) *Nitrite plus Nitrate.*—The standard phenoldisulphonic acid method was employed, modified to remove the interference by chloride which occurs at depth in these soils. One ml of percolate was transferred to a 15 ml centrifuge tube and 0.2 ml 0.045N silver sulphate solution added with shaking, followed by 0.15 ml 0.1N sodium hydroxide and 1.65 ml water. This treatment removes chloride and precipitates organic matter. After mixing and centrifuging, 1.5 ml of the supernatant liquid was transferred to a 50 ml beaker, acidified with 0.15 ml 0.1N sulphuric acid, treated with 0.25 ml of 6 per cent. A.R. hydrogen peroxide, and allowed to stand for 15 min. After neutralizing the acid solution with the calculated quantity of alkali it was evaporated to dryness, cooled, and treated with phenoldisulphonic acid. The diluted solution was then treated with excess alkali, cooled, made to volume, and filtered through a Whatman 541 paper before photometric comparison. The method tends to give high blank values (equivalent to about 5 μg nitrogen/ml) due probably to colloidal silver oxide; values are, however, quite reproducible.

(ii) *Nitrite.*—The method of Rider and Mellon, as described by Barnes and Folkard (1951) was employed. By subtracting nitrite nitrogen from total oxidized nitrogen the value for nitrate nitrogen was obtained.

III. Results

All results are reported in milligrams of nitrogen (as nitrite or nitrate ion) per kilogram of air-dry soil.

(*a*) *Percolation with Ammonium Sulphate*

Miyake (1916) and Lees and Quastel (1946*a*) have described the autocatalytic nature of the nitrification process in the presence of excess substrate. The curve usually obtained has the form

$$\log(y/A - y) = K(t - t_m),$$

where y = nitrate nitrogen accumulated in time t,
A = asymptotic value of y,
t_m = time of half-completion,
K = rate constant.

Martin (1949) utilized the fact that K is actually a regression of $\log(y/A - y)$ on time and, by means of a regression analysis, attempted to compare statistically the rate constants obtained from several percolation tests. However, a more detailed mathematical analysis (Cox, unpublished data) showed that a family of sigmoid curves can be obtained from a

Table 1

TIME FOR NITRATE NITROGEN TO REACH CERTAIN VALUES

Mean of duplicate determinations

Reaction (*a*): $NH_4^+ + 3O \longrightarrow NO_2^- + H_2O + 2H^+$
Reaction (*b*): $NO_2^- + O \longrightarrow NO_3^-$

Depth (in.)	Time for Nitrate Nitrogen to Reach			
	50 Mg/Kg Soil		Half Maximum	
	(*a*)	(*b*)	(*a*)	(*b*)
0-3	1.7	1.9	7.5	8.9
3-6	5.0	6.6	12.3	16.8
6-12	9.6	11.6	19.2	23.0
18-24	12.9	23.5	17.7	30.3
30-36	12.2	32.6	16.0	35.0

single rate constant. Each curve shows the same value for the tangent at the point of inflexion but the relative displacement of each point of inflexion along the time axis varies with the choice of the initial rate of the process. The regression lines obtained from the curves are all identical in slope but show different intercepts on the $\log(y/A - y)$ axis at zero time. It therefore seems that this approach may have theoretical limitations, and since none of these curves could be fitted to a simple expression the rates of nitrification under different treatments have been compared quite empirically. Figure 1 shows typical results from three selected

depths and Table 1 shows the respective rates of transformation of ammonia to nitrite and of ammonia to total oxidized nitrogen. The lag phase was arbitrarily taken as the time (in days) from the start of percolation to the time at which 50 mg nitrogen/kg soil (as nitrite or nitrate) had been accumulated by each sample. The time of half-completion

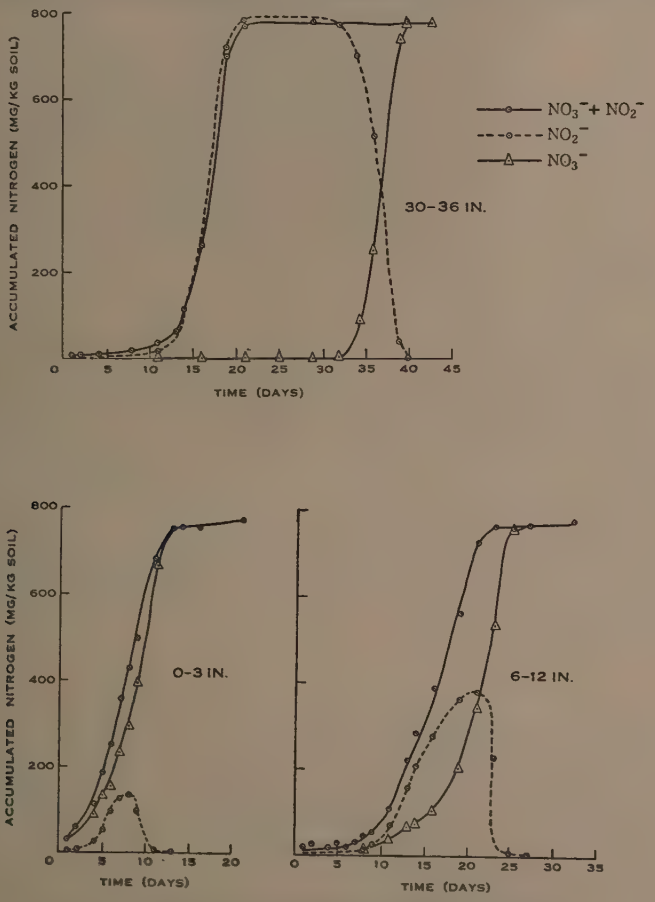

Fig. 1.—Relation of nitrifying capacity to depth.

was obtained from the point of inflexion of each curve, using half the maximum value of nitrate nitrogen finally attained in each unit (this varied between 730 and 790 mg/kg).

Reaction (*a*) in Table 1 is the first stage in the nitrification process, and values for this were taken from the curve for total oxidized nitrogen (not given) since this fraction is present as, or has been through, the nitrite stage. It appears therefore that, firstly, rates of nitrification decrease markedly with depth; and, secondly, the speed of the first stage

becomes roughly constant at depths below 12 in. but the subsequent rate of oxidation of nitrite becomes increasingly slower. This is reflected in the maximal nitrite nitrogen recorded over several days in soil percolates from the 18-24 and 30-36 in. depths. Even at the surface, however, appreciable quantities of nitrite appear before further oxidation takes place.

(*b*) *Repercolation Tests*

Following the initial percolation with ammonium sulphate, one sample from each depth was repercolated with five successive 120 ml portions of 0.01N ammonium sulphate. Between percolations, the soil columns were washed free of nitrate and about 0.5 g calcium carbonate added to each. The correct concentration of substrate was maintained in each unit by adding 10 ml 0.12N ammonium sulphate to the reservoir and making up the weight deficit with distilled water.

First repercolations always resulted in a more rapid oxidation of ammonia; at each depth, however, the curves displayed the original shape but were more condensed along the time axis. Successive repercolations then gave rise to linear transformations which, as shown by Lees and Quastel (1946*b*) suggest conditions of bacterial saturation. Nitrite was observed in all percolates, even after five repercolations, in some cases reaching momentarily high values during early stages of the first repercolation. The average time of completion of the reaction from ammonia to nitrate after bacterial saturation had been reached was just over 2 days at all depths. Thus the cause of the differences in nitrification rates and of high nitrite accumulation gradually disappeared on enrichment of the samples.

(*c*) *The Effect of Chloride and Azide*

The relative slowness of nitrite oxidation, compared with the primary stage of ammonia oxidation, may be due to a real difference in bacterial activity or numbers with depth. It may also be caused by some specific soil condition which inhibits, in a partially selective manner, the biological oxidation of nitrite. Of many possibilities, there are two factors either or both of which could contribute to this effect in these soils, namely, the rises in chloride concentration and in pH value with depth. Percolation of surface samples with ammonium sulphate solution containing chloride (at the same concentration that would have occurred during percolation of the 30-36 in. layer) failed to cause any marked differences except that nitrite persisted longer than, but did not reach such high levels as, in the series without added chloride. Similarly, percolation of the 30-36 in. sample, which had been washed free of chloride, showed no change in the rate of ammonia oxidation but nitrite levels remained at their maximum values for a greatly reduced period, resulting in a more rapid accumulation of nitrate than in the unwashed sample.

That nitrite oxidation was wholly biological at all depths was confirmed by percolation of 0.01N sodium nitrite in presence of 0.001N sodium azide. After 35 days, 86 per cent. of the nitrite originally added remained unoxidized in the 0-3 in. sample, while at 30-36 in., 97 per cent. of added nitrite was still present at the end of this time.

(*d*) *Effect of pH*

Twelve 20 g portions of surface soil were placed into detached soil percolator columns as previously described. Six were washed with 120 ml 0.01N sulphuric acid and then leached free of acid with two washings with

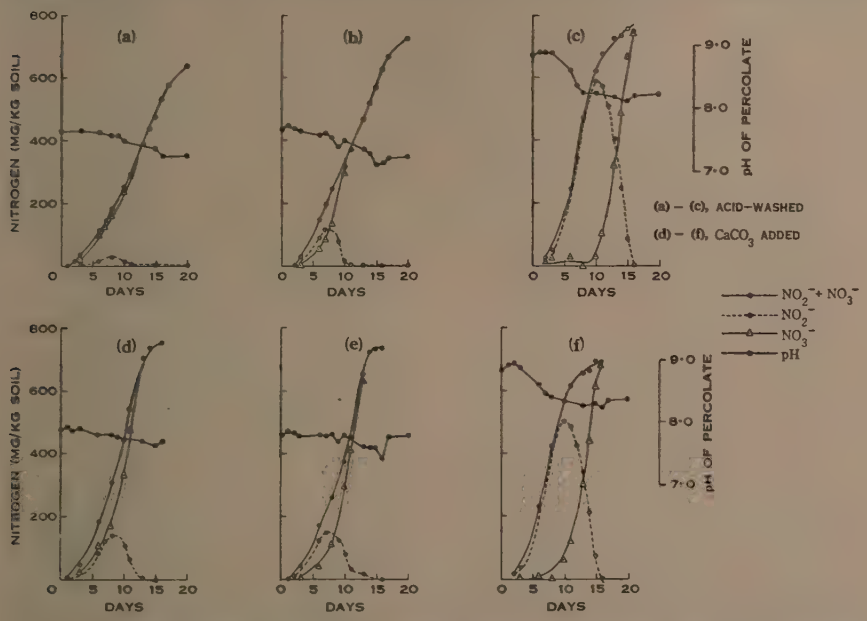

Fig. 2.—Accumulation of oxidized nitrogen fractions in relation to pH of percolate. (*a*) and (*d*), controls; (*b*) and (*e*), 4.8 m-equiv. Na_2SO_4 present; (*c*) and (*f*), 4.8 m-equiv. Na_2CO_3 present.

distilled water. The remainder were each treated without previous acid washing with 0.5 g portions of calcium carbonate. All units were then assembled and percolated with 120 ml of 0.01N ammonium sulphate containing varying proportions of sodium sulphate and sodium carbonate such that, while the concentration of sodium ion was maintained at 4.8 m-equiv./unit, the ratio between sulphate and carbonate ions varied. Soil samples were thus percolated with liquids which were constant in ammonium ion and ionic strength but varied in pH. As controls, one acid-washed soil was percolated with pure ammonium sulphate solution and one unwashed sample with this solution after addition of calcium

carbonate. The pH values of the percolate were determined in each reservoir at each sampling period by means of a glass electrode. Using the assembly described by Raupach (1954), with the internally fitted ground glass junction, diffusion of chloride into the percolating fluid was minimized. At the end of the experiment, pH values were determined both on the wet soil paste from each unit and, after moisture determination on the paste, on a 1 : 5 water suspension.

Figure 2 shows some of the curves obtained; pH values of the percolates at each sampling are also recorded. The course of the transformation was markedly affected by the alkalinity of the percolating solution. The presence of calcium carbonate (with no other salts added)

TABLE 2
pH VALUES OF PERCOLATES AT WHICH NITRITE ATTAINS A MAXIMUM

Treatment		Maximum Nitrite Ion (mg N/kg)	pH of Percolate	Final pH of Soils	
Sodium Sulphate (m-equiv.)	Sodium Carbonate (m-equiv.)			Paste	1 : 5 Suspension
Acid-washed					
0.0	0.0	22	7.6	7.15	7.65
4.8	0.0	112	7.6	7.18	7.72
3.6	1.2	216	7.7	7.25	7.93
2.4	2.4	384	7.9	7.50	8.15
1.2	3.6	461	8.0	7.65	8.40
0.0	4.8	587	8.2	7.67	8.65
Unwashed + 0.5 g calcium carbonate					
0.0	0.0	140	7.8	7.45	7.95
4.8	0.0	149	7.8	7.55	8.05
3.6	1.2	239	7.8	7.56	8.17
2.4	2.4	248	8.0	7.57	8.32
1.2	3.6	465	8.1	7.69	8.48
0.0	4.8	504	8.3	7.85	8.72

caused an increased rate of ammonia oxidation (cf. Figures 2*d* and 2*a*) and apparently the presence of sodium sulphate alone (Figure 2*b*) had the same effect. Increasing the pH of the percolate caused a progressive increase in ammonia oxidation with a simultaneous reduction in the rate of transformation of nitrite, reflected in the increased amounts of this fraction found in the solutions. In all units, pH values declined throughout the experiment except for a slight but unexplained rise just before completion. It appears from the curves that the build-up of nitrite continues until the pH of the percolating solution reaches a certain value. Table 2 shows the pH values in each treatment at which the accumulation of nitrite reaches a maximum, with the actual values of nitrite nitrogen attained at that time. It is clear that, with increasing alkalinity, there is an increase in accumulated nitrite which approaches the levels formed in

percolating samples from the 18-24 in. depth in the absence of salts. pH values of each soil at the end of this experiment are also recorded in Table 2. Both series show the expected increase of soil pH with increasing alkali treatment (whether calcium carbonate or sodium carbonate) which is correlated with the final pH of each percolate. It was, of course, impossible to obtain actual soil pH values during percolation and we can only assume that treatment with alkali did in fact cause parallel variations in soil pH. Our results suggest, therefore, that the principal cause of nitrite accumulation by subsoil horizons in the percolators is a selective inhibition of the nitrite-oxidizers by high alkalinities.

TABLE 3
RELATION OF pH TO MAXIMUM NITRITE ACCUMULATED IN ENRICHED SOILS

Treatment	Maximum Nitrite Ion (mg N/kg soil)	pH of Percolate
Calcium carbonate alone:		
First repercolation	84	7.6
Second repercolation	21	7.9
Fourth repercolation	24*	8.3*
Calcium carbonate + 4.8 m-equiv. sodium carbonate:		
First repercolation	197	8.6
Second repercolation	80	8.8

* 4.8 M-equiv. sodium carbonate added.

Some degree of adaptation on the part of the soil organisms is apparent from the detailed curves. In soils treated only with sodium sulphate, pH values did not rise above 7.7; in the most alkaline treatment the lowest pH recorded was 8.1 and in spite of this the disappearance of nitrite was eventually rapid. To test the adaptability of the organisms to pH stress, two soils were percolated in the presence of calcium carbonate with, respectively, pure 0.01N ammonium sulphate solution and 0.01N ammonium sulphate containing 4.8 m-equiv. sodium carbonate, and were then repercolated with similar solutions. Table 3 shows the maximum nitrite accumulated, with the corresponding pH values at each sampling. With successive repercolations, the pH values at maximum nitrite content continued to rise and the maximum nitrite value fell off sharply. It is clear, therefore, that the initial sensitivity of the organisms to pH values over 7.5 is overcome by successive enrichment with solutions of higher pH. The addition of sodium carbonate to the fourth repercolation of soil previously treated without this compound produced a further rise in pH at maximum nitrite content, while the nitrite level reached was scarcely affected.

IV. Discussion

The experiments described were carried out with the air-dried soils over a period of about 12 months. Percolation tests performed after 5 months' storage revealed no differences in activity, and it is assumed that the soil samples remained unaffected for the rest of the experimental period.

Inspection of the individual curves of the samples taken at various depths showed that, in spite of the marked increase in lag period, the final shapes of the logistic portion of each curve were similar (see Fig. 1). This implies that after the initial stimulation given by the presence of active metabolite in the percolating fluid the proliferation of active cells over the soil mass takes place at a constant rate, and that the relative efficiencies of the nitrifiers at this stage of the process are probably similar at each depth. If this assumption is valid then the increases in length of the lag periods are caused by decreases in numbers of these organisms with depth. This has been shown for other types of organisms in soils (e.g. Newman and Norman 1943). These observations add weight to the suggestion in paper I of this series that nitrate found below 1 ft in fallow soils of this type is the result of leaching rather than active nitrification.

The differential effect of pH on the two stages of the nitrification process has been studied by other workers. Martin, Buehrer, and Carter (1942) made a comparative study of the oxidation of ammonium salts and urea in desert soils from Arizona using an incubation method; they found appreciable accumulation of nitrite even in well-aerated samples, for periods of time depending on the degree of alkalinity above a critical threshold soil pH value of 7.7. Drouineau, Gouny, and Lefèvre (1948) found that highly calcareous soils accumulated nitrite under incubation to a greater extent than slightly calcareous soils, and suggested that calcium carbonate itself modified the relative rates of oxidation of ammonia to nitrite.

No evidence has been found in the literature that the chloride ion itself selectively inhibits the nitrite-oxidizers, but excessive chloride has been shown to retard the formation of nitrate, e.g. Hahn, Olson, and Roberts (1942) found that potassium chloride inhibited *Nitrosomonas* but not *Nitrobacter*; Butovskii (1950) and Yankovitch and Yankovitch (1953) have presented similar results. It is clear from the results presented that, although the amount of chloride in the deepest sample was relatively low (0.12 per cent. sodium chloride on oven-dry basis) the accumulated nitrite was more rapidly oxidized when the sample was previously washed free of salts. However, one cause of excessive nitrite formation in the percolators is shown to be high pH, in agreement with the results of Martin, Buehrer, and Caster (1942). Decrease in nitrifying capacity in the field with depth, ascribed to a decrease in numbers, may therefore be further enhanced by a partially selective inhibition of the

nitrite-oxidizers by high pH values, but confirmation of this hypothesis would need further field work on the amounts of nitrite nitrogen present in these soils.

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THE ARTIFICIAL INSEMINATION OF THE MERINO SHEEP FOLLOWING THE SYNCHRONIZATION OF OESTRUS AND OVULATION BY PROGESTERONE INJECTED ALONE AND WITH PREGNANT MARE SERUM GONADOTROPHIN (PMS)

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Summary

Two groups (2 and 3), each of 112 stud Merino ewes, which were experiencing regular oestrous cycles, received daily injections of 10 mg progesterone for 16 days, in an attempt to synchronize oestrus and ovulation for artificial insemination. One group (3) received an additional injection of 500 i.u. pregnant mare serum gonadotrophin (PMS) the day after the final progesterone injection. Injections were staggered so as to bring between 15 and 20 ewes of each group into oestrus on each of six days, on which days approximately equal numbers of untreated oestrous ewes were inseminated (group 1).

In group 2 (progesterone alone), 104 ewes were teased and inseminated within 4 days of the final injection, including 74 (71 per cent.) on the third day. Corresponding numbers of ewes for group 3 (progesterone-PMS) were 107, 96 (90 per cent.). Injection of PMS significantly advanced and improved the precision of time of onset of oestrus, and presumably of ovulation.

Of 315 ewes inseminated in all groups, 265 received 0.10 ml undiluted semen; 33, 0.05 ml; and 17, 0.20 ml. There were no apparent differences in lambing rate.

Ewes inseminated, ewes lambing, and lambs born were: group 1, 104, 58 (55 per cent.), 74 (71 per cent.); group 2, 104, 53 (51 per cent.), 64 (62 per cent.); group 3, 107, 61 (57 per cent.), 85 (79 per cent.); overall, 315, 172 (55 per cent.), 223 (71 per cent.).

There were no significant differences between groups in the proportion of ewes which lambed, or in the twinning rate.

Ewes in oestrus and inseminated 72 hr after the final injection of progesterone had a significantly higher lambing rate than did ewes inseminated a day later.

It is concluded that progesterone, particularly if used in conjunction with PMS, is of value in synchronizing oestrus in the breeding season for precisely planned artificial insemination.

I. Introduction

Major factors mitigating against the widespread use of artificial insemination of sheep in Australia are, firstly, the relatively poor conception rates likely to be obtained when diluted or stored semen is used (Keast and Morley 1949; Emmens and Blackshaw 1955); secondly, the

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unpredictability of the number of ewes expected in oestrus on any one day; and, thirdly, the period of observation and insemination—3 or more weeks —needed to cover all ewes in a flock. The production, at will, of oestrus and ovulation accompanied by a high degree of fertility would eliminate these three factors. Fresh semen could be used, the numbers of sheep to be inseminated adjusted to meet the expected supply of semen—keeping one or more rams in reserve—and the whole period of insemination considerably shortened.

Attempts to achieve this by the induction of synchronized oestrus and ovulation during anoestrus, using progesterone and pregnant mare serum (PMS), have been largely unsuccessful (Robinson 1955). While oestrus with ovulation can readily be induced, fertility following insemination is low owing to an interaction of factors affecting both the ram and the ewe, the additive effect of which is to reduce fertility to below an effective level.

Ovulation in many species can be inhibited by the injection of progesterone. Makepeace, Weinstein, and Friedman (1937), Everett (1948), and Rothchild and Fraps (1949) have shown that ovulation can be temporarily suppressed in the reproductively active rabbit, rat, and chicken, respectively. Dutt and Casida (1948) and O'Mary, Pope, and Casida (1950) showed that ovulation could be suppressed by the daily injection of 10 mg progesterone to sheep undergoing regular oestrous cycles. Seventy per cent. of treated animals came into oestrus on the third day after cessation of treatment. Fertility to natural service was normal. Hunter (1954) obtained essentially similar results, but concluded that for really precise work, such as ovum transplantation, a considerable margin of error should be allowed in the number of ewes treated, to allow for variations in the time of onset of oestrus after the withdrawal of progesterone treatment.

Ovulation following progesterone withdrawal is brought about by the secretion of gonadotrophin by the anterior pituitary. The ewe is highly reactive to PMS injected in the late progestational phase of the normal cycle. In addition to multiple ovulation, such treatment advances the time of ovulation and onset of oestrus without any impairment of fertility (Robinson 1951). This suggests that the injection of a small dose of PMS a short time after progesterone withdrawal might accelerate and perhaps standardize the time of ovulation.

This paper reports the results of an experiment conducted on the Old Cobran Merino Stud, Caldwell, N.S.W., in which 315 stud Merino ewes, in three groups, were artificially inseminated in the normal breeding season. One group was a control flock of sheep which came into oestrus in the normal manner. Oestrus and ovulation were controlled in a second group by the daily injection of progesterone, and in the third group by progesterone followed by a single injection of PMS.

II. Material and Methods

(*a*) *Experimental Animals*

A flock of 373 stud Merino ewes of ages varying from 1½ to 6 years was available. Included were 105 maiden ewes. Oestrus was detected by 6 vasectomized teaser rams, and ewes were inseminated with semen from 3 stud Polled Merino rams.

(*b*) *Management and Observations*

The teasers were joined on February 1, 1955. They were raddled on the brisket daily with L.B.E. branding fluid, and daily records of service

Table 1

Injection and insemination schedule, groups 2 and 3,* 1955

Group 2, progesterone (10 mg/day for 16 days)

Group 3, progesterone (10 mg/day for 16 days + 500 i.u. PMS)

P, progesterone (10 mg/day); PMS, pregnant mare serum (500 i.u.); I, inseminate

Planned date of insemination:	Mar. 16		Mar. 17		Mar. 18		Mar. 19		Mar. 20		Mar. 21	
Group:	2	3	2	3	2	3	2	3	2	3	2	3
No. of ewes:	19	19	19	19	19	19	18	18	18	18	19	19
Schedule:												
Feb. 26, 1955	P	P										
Feb. 27	P	P	P	P								
Feb. 28	P	P	P	P	P	P						
Mar. 1	P	P	P	P	P	P	P	P				
Mar. 2	P	P	P	P	P	P	P	P	P	P		
Mar. 3 to	P	P	P	P	P	P	P	P	P	P	P	P
Mar. 13	P	P	P	P	P	P	P	P	P	P	P	P
Mar. 14	—	PMS	P	P	P	P	P	P	P	P	P	P
Mar. 15	—	—	—	PMS	P	P	P	P	P	P	P	P
Mar. 16	I†	I†	—	—	—	PMS	P	P	P	P	P	P
Mar. 17			I	I	—	—	—	PMS	P	P	P	P
Mar. 18					I	I	—	—	—	PMS	P	P
Mar. 19							I	I	—	—	—	PMS
Mar. 20									I	I	—	—
Mar. 21											I	I

* Group 1 were untreated controls. As many ewes as were teased were inseminated on March 16-22.

† These were *planned* dates of insemination. Not all ewes had been teased by these dates. See Table 2 for actual numbers of ewes inseminated on each day.

were kept. Inseminations were planned for March 16-22 inclusive, during which period a high proportion of ewes teased between February 10 and 17 could be expected to be in oestrus again. Consequently such ewes—149 in number—were drafted into the control group (group 1). The remainder —224 in number—were randomized into groups 2 and 3, each of 112 ewes.

TABLE 2

NUMBERS OF EWES INSEMINATED FROM EACH OF THREE RAMS DAILY, 1955

Date of Insemination	Group 1 Controls, Untreated				Group 2 Progesterone				Group 3 Progesterone-PMS				Pooled Totals*
	Ram P	Ram O	Ram B	Total	Ram P	Ram O	Ram B	Total	Ram P	Ram O	Ram B	Total	
Mar. 16	8	7	8	23	3	3	7	13	5	7	5	17	53
17	4	7	6	17	8	5	7	20	4	8	5	17	54
18	7	4	0	11	4	8	0	12	15	5	0	20	43
19	3	6	4	13	2	4	10	16	2	10	4	16	45
20	4	8	4	16	6	4	10	20	5	9	5	19	55
21	0	11	4	15	5	7	8	20	2	7	7	16	51
22	9	0	0	9	3	0	0	3	2	0	0	2	14
Total:	35	43	26	104	31	31	42	104	35	46	26	107	315

* All treatments, all rams.

Group 2 received 10 mg progesterone in peanut oil (10 mg/ml) intramuscularly, between 8 and 9 a.m. daily for 16 days. Group 3 received similar treatment, followed between 12 noon and 1 p.m. the day after the final progesterone injection by 500 i.u. PMS, subcutaneously in 0.9 per cent. saline.

Injections were staggered in accordance with the schedule shown in Table 1, so that treatment commenced and finished for 18 or 19 ewes of each group, on each of six successive days. The intention was to bring these numbers of animals from these groups into oestrus on each day and to inseminate them, together with as many of the untreated ewes of group 1 as were teased. Table 2 presents details of ewes inseminated and rams used daily. The number of ewes inseminated with the semen of each ram depended on the volume available, and on which of the teased ewes it was desired to join to the ram for stud purposes.

TABLE 3

SUMMARY OF SEMEN APPRAISALS

Dates of collection, Mar. 16-22 inclusive

Ram	Semen Characteristics						Remarks
	Colour	Volume (ml)	Density	Motility	pH	% Live Sperm*	
P	Ivory	1.8 ± 0.3	5	5	6.7 ± 0.4	95, 96	
O	Ivory	2.2 ± 0.3	4½-5	4½-5	6.7 ± 0.2	96, 96	
B	Ivory	2.1 ± 0.2	5	5	6.6 ± 0.3	92, 93	Urinated in one sample

* Estimates made on first and last days of insemination.

Six vasectomized teaser rams were with the ewes throughout the insemination period. Examinations for service were made at 6.30 p.m. each day, and at 7 a.m. the following day when teased ewes were drafted for insemination. Time of onset of oestrus was classed into intervals of 12 hr. This involved a slight error as the actual times of observations for oestrus were separated by intervals of 11½ and 12½ hr each day.

(*c*) *Semen Collection and Examination*

Semen was collected daily from each ram using a bipolar rectal stimulator (Blackshaw 1954). Volume, colour, and pH, as assessed by a spot plate-indicator technique, were recorded and estimates made of density and motility, using a score range of 0-5 (Perry 1952). A congo-red–nigrosine live-dead stain preparation (Blackshaw 1955) was made of samples collected on the first and last days of insemination.

Table 3 presents a summary of semen characteristics.

Fig. 1.—Ewe in revolving inseminating crate. (*a*) Crate in loading position. (*b*) Crate in inseminating position. Inseminator's seat is in the foreground.

(*d*) *Insemination*

Ewes were inseminated in a portable revolving AI crate set up indoors on the shearing board (Figs. 1(*a*) and 1(*b*)). Forty ewes could be

inseminated per hour using a team of four men, including the inseminator. Ewes were inseminated once only, between 9 and 11.30 a.m.

Undiluted semen was used. A dose of 0.1 ml was used for 265 ewes, but where there was an excess of semen 0.2 ml was used (17 cases), and where there was a shortage 0.05 ml was used (33 cases).

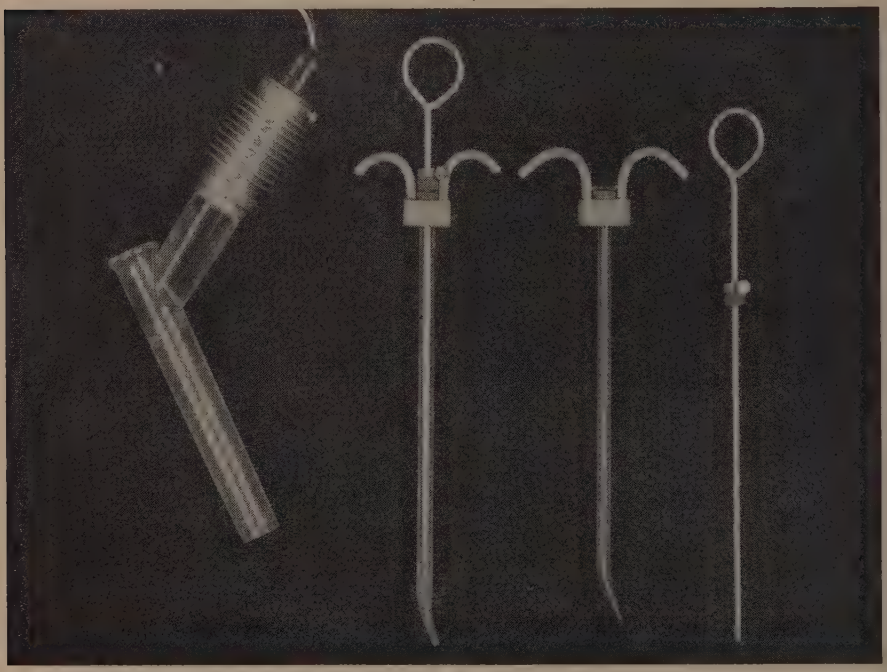

Fig. 2.—Inseminating syringes and "Perspex" illuminated speculum. The syringes are constructed of shortened 2 ml graduated pipettes with a metal plunger, on the distal end of which is a rubber piston ring. The finger grip on the pipette is removable. The plunger is notched, the distances between notches corresponding to a delivery of 0.1 ml. A rubber cushioned set-screw mounted thereon permits delivery of this volume, or of a fraction or multiple of it.

Special inseminating syringes and speculum were made in the University Physiology School workshop and are illustrated in Figure 2. The syringes were made of 2-ml graduated pipettes, cut short and belled out at the wide end, and curved at the tip. The piston consisted of a length of welding flux, electroplated, and with a piston ring groove turned on the distal end. The piston ring was a 5 mm length of bicycle valve rubber. The proximal end of the piston was notched, the distance between notches representing a delivery of 0.1 ml, and a rubber cushioned set-screw mounted thereon. A rubber-mounted removable finger grip was mounted on the

syringe. The comfortable span of these syringes was 0.6 ml, sufficient to inseminate six sheep with 0.1 ml or 12 sheep with 0.05 ml. There was virtually no waste, and 19 sheep could regularly be inseminated, each with 0.1 ml, from a 2 ml sample of semen, after routine semen examinations. The "Perspex" speculum was illuminated by a 6 V globe run off an accumulator.

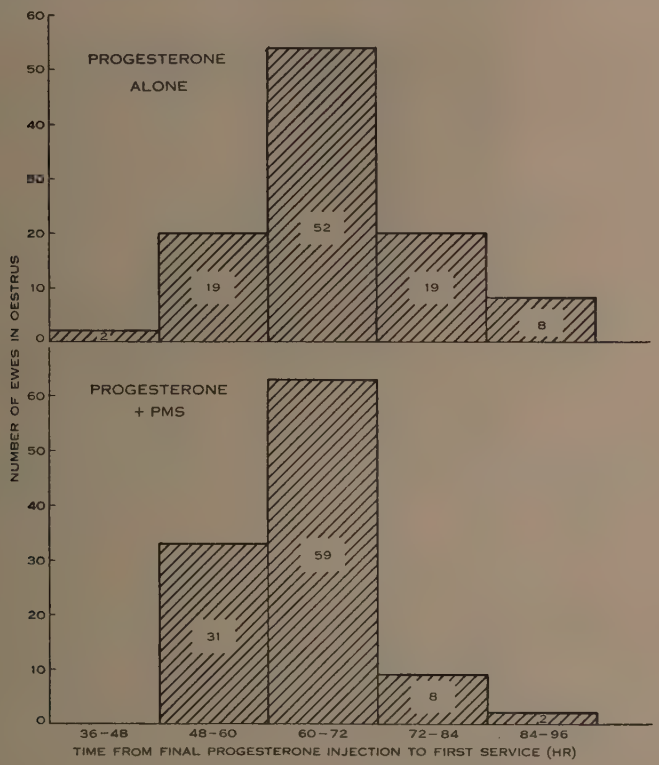

Fig. 3.—Distribution of time of onset of oestrus after the final injection of progesterone to groups 2 and 3. Group 2, progesterone alone (10 mg/day for 16 days). Group 3, as for group 2, plus 500 i.u. PMS the day after the final injection of progesterone. Comparison of ewes served "early" (i.e. within 72 hr) and ewes served "late" (i.e. after 72 hr) between groups 2 and 3: $\chi^2 = 8.63$; d.f. $= 1$; $P < 0.01$. Numbers on columns represent percentage response in each interval.

After each ewe was inseminated the speculum was cleaned with a swab soaked in saline and "Zephiran", and the outside of the inseminating syringe wiped over.

All semen was used within 1 hr of collection and no decline in motility was apparent during the period from collection to final insemination.

TABLE 4

SUMMARY OF TREATMENTS, INSEMINATIONS, AND LAMBINGS

Group	Treatment	Number of Ewes		Number of		Ewes Lambing as Percentage of		Lambs Born as Percentage of			Pregnancies*	
		Treated	Inseminated	Ewes Lambing	Lambs Born	Ewes Treated	Ewes Inseminated	Ewes Treated	Ewes Inseminated	Ewes Lambing	Singles	Twins
1	Control	149†	104	58	74	39	55	50	71	128	42	16
2	Progesterone	112	104	53	64	47	51	57	62	121	42	11
3	Progesterone-PMS	112	107	61	85	54	57	76	79	139	37	24
	Total:	373	315	172	223	46	55	60	71	130	121	51

* Comparison of twinning rate between groups: $\chi^2 = 4.85$; degrees of freedom $= 2$; $0.1 > P > 0.05$; not significant.
† Ewes teased two cycles earlier and likely to be in oestrus during the week of inseminations.

(e) *Post-insemination Treatment and Observations*

Raddled vasectomized teaser rams were joined 1 week after insemination, and returns to service recorded. Ewes were lambed down in a 40 acre homestead paddock and lambs recorded and mothered daily. Of 223 lambs born, the parentage of only eight, born dead, was in any doubt.

III. Results

(a) *Suppression of Oestrus by Progesterone*

No ewe exhibited oestrus during the period of progesterone treatment. Oestrus cycles were prolonged up to a length of 31 days.

(b) *Oestrus after Withdrawal of Progesterone*

Figure 3 shows the distribution of times of onset of oestrus after cessation of progesterone injections. Of 112 ewes treated in each of groups 2 and 3, 104 and 107, respectively, were teased within 4 days. The injection of 500 i.u. PMS the day after the final progesterone treatment gave an earlier and more predictable time of onset of oestrus than did progesterone withdrawal alone ($P < 0.01$).

(c) *Returns to Service*

Of 315 ewes inseminated, 126 (40 per cent.) returned to service by the vasectomized rams one cycle later. There was no significant difference between groups.

(d) *Factors Affecting Lambing Results*

(i) *Treatment.*—Table 4 presents a summary of lambing results. Of 315 ewes inseminated, 172 lambed yielding 233 lambs, including eight born dead. There was no significant difference between groups in the proportion of ewes which lambed. The apparently greater rate of twinning in group 3 (progesterone-PMS), while suggestive, was not statistically significant.

(ii) *Day of Insemination.*—There was no significant day-to-day variation in the proportion of successful inseminations.

(iii) *Age of the Ewe.*—Maiden ewes responded in essentially the same manner as did mature animals (Table 5). The apparently greater twinning rate of the mature animals, while suggestive, was not statistically significant.

(iv) *Volume of Semen.*—Relatively few ewes received other than 0.1 ml undiluted semen. The limited data suggest no effect of the volume of semen (Table 6).

(v) *The Ram.*—The three rams were used as evenly as possible between days and groups. One of them (ram B) appeared to be signifi-

cantly more fertile than the others ($P < 0.05$; Table 7). There was no evidence of this from the routine sperm examinations (see Table 3).

(vi) *Time of Insemination Relative to Onset of Oestrus.*—As ewes were inseminated once daily, commencing 1 or 2 hr after teased ewes had been drafted and continuing for some 2-3 hr, the range of time from the

TABLE 5

LAMBING AND TWINNING RATES IN MATURE AND MAIDEN EWES

Pooled data from groups 1, 2, and 3

Class of Ewe	Ewes Inseminated	Ewes Lambing		Lambs Born	Pregnancies	
		No.	%		Singles	Twins
Maiden	93	48	52	57	39	9
Mature	222	124	56	166	82	42
Total:	315	172	55	223	121	51

Comparison between maiden and mature ewes of:
Ewes which lambed: not significant
Proportion of twins: $\chi^2 = 3.10$; degrees of freedom $= 1$; $0.1 > P > 0.05$; not significant

onset of oestrus to insemination was from about 2 to 27 hr. This could conceivably affect conception rates. Table 8 presents a summary of data on lambing rates relative to time from the onset of oestrus to insemination. There was no significant difference in lambing rates between ewes teased within the limits of 27 to 15 hr and 15 to 2 hr before insemination, in any group.

TABLE 6

LAMBING RATES IN RELATION TO THE VOLUME OF UNDILUTED SEMEN USED FOR ARTIFICIAL INSEMINATION

Volume of Semen (ml)	Ewes Inseminated	Ewes Lambing	Percentage Lambing
0.05	33	18	55
0.10	265	144	55
0.20	17	10	58
Total:	315	172	55

(vii) *Time of Onset of Oestrus Relative to Final Progesterone Injection.*—In groups 2 and 3, of 211 ewes which came into oestrus after progesterone withdrawal, 172 did so and were inseminated within 72 hr of the final injection of progesterone, and 103 lambed. Of the remaining 39 which were teased, and inseminated at the most 48 hr later, only 11 lambed. This difference in lambing rate is highly significant ($\chi^2 = 12.8$; d.f. $= 1$; $P < 0.01$).

(e) *Lambing Dates*

The mean gestation period for single pregnancies was 149.6 ± 1.9 days, and for twins, 148.7 ± 1.7 days. All 172 ewes lambed within the 14-day period August 10 to 23; 165 of them between August 10 to 19 (Fig. 4).

TABLE 7

LAMBING RATES AS AFFECTED BY THE SIRE
Pooled data from groups 1, 2, and 3

Ram	Ewes Inseminated	No. of Inseminations		Lambs Born	Inseminations Successful (%)	Twin Pregnancies (%)
		Succeeding	Failing			
P	101	52	49	66	54	27
O	120	58	62	73	48	26
B	94	62	32	84	66	35
Total:	315	172	143	223	55	30

Comparisons between rams of:
Ewes which lambed: $\chi^2 = 7.21$; degrees of freedom $= 2$; $P < 0.05$
Proportion of twins: not significant

IV. DISCUSSION

Although there appears to be a slight but significant difference in the fertility of the three rams used in this trial, the distribution of their semen between groups and days of insemination is sufficiently uniform to largely eliminate this factor in discriminating between various factors affecting the lambing rate.

TABLE 8

LAMBING RATES IN RELATION TO TIME OF ONSET OF OESTRUS RELATIVE TO INSEMINATION
Day 0 = day of insemination; day —1 = day before insemination. Inseminations made between 9 and 11.30 a.m. on day 0

Time of Onset of Oestrus Relative to Insemination	Group	Number of Ewes	Number Lambing	Per Cent. Lambing
7 a.m. to 6.30 p.m. on day —1 (*c.* 15 to 27 hr before insemination)	1	44	23	52
	2	40	18	45
	3	42	23	55
	Total:	126	64	51
6.30 p.m. on day —1 to 7 a.m. on day 0 (*c.* 2 to 15 hr before insemination)	1	60	35	58
	2	64	35	55
	3	65	38	58
	Total:	189	108	57

Comparison of lambing rate between times of onset of oestrus: not significant

The overall lambing rate of 55 per cent. of ewes inseminated conforms with results to be expected in the sheep. The overall lambing percentage of 71, expressed on the basis of ewes inseminated, is rather higher than usual and undoubtedly is related to the fact that inseminations were made in late March when the Merino sheep is approaching the peak of its reproductive efficiency (Watson 1953). It is tempting to attribute the higher proportion of twins in group 3 to PMS-induced multiple ovulations, but this will not stand up to critical statistical examination.

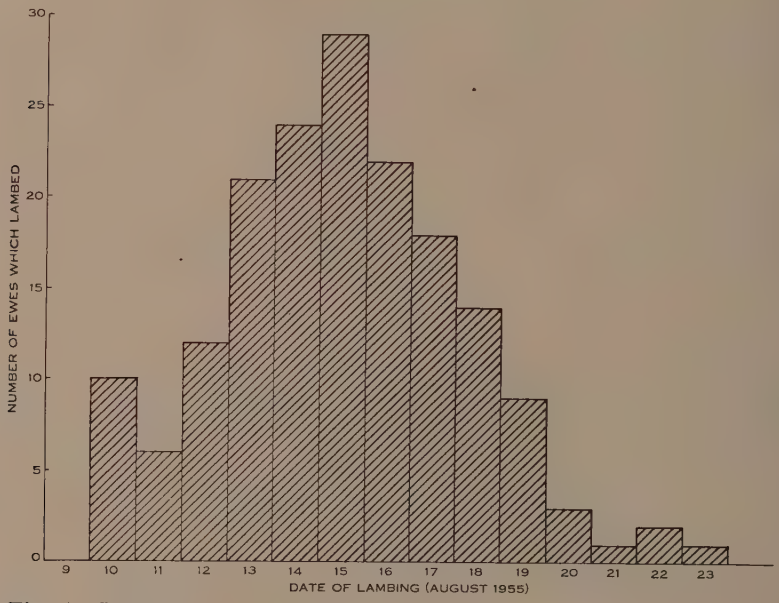

Fig. 4.—Distribution of lambing dates of 176 ewes which lambed as the result of artificial insemination between March 16 and 22 inclusive. Pooled data from groups 1, 2, and 3.

It is apparent that the suppression and subsequent release of ovulation by progesterone injection and withdrawal has no deleterious effect on subsequent fertility to artificial insemination. This conforms to the finding of O'Mary, Pope, and Casida (1950), for natural service, but is considerably more conclusive in view of the much larger numbers of animals involved. It is in contrast to the observation of Baker *et al.* (1954) and of Trimburger and Hansel (1955), who obtained almost complete infertility in sows and cows, respectively, at the delayed oestrus which followed progesterone withdrawal. Earlier, Willett (1950) had obtained 11 pregnancies from 22 services to heifers after progesterone withdrawal. Hence it is by no means established that normal fertility cannot be obtained in the cow at the delayed oestrus following progesterone withdrawal.

Trimburger and Hansel (1955) consider that one factor involved may be the time of commencing progesterone treatment relative to the normal cycle. This view receives some support from the observation of Brock and Rowson (1952) that ova degenerate in cows in which oestrus and ovulation is delayed for more than 5 days after PMS treatment. As far as the sheep is concerned, this argument does not apply. There was no evidence of any interaction between the length of the prolonged dioestrus and subsequent lambing rate. It is possible, therefore, to enter a flock in the breeding season and, without knowing anything of the cycle of any individual ewe, to commence progesterone injections, continue them for 16 days—long enough to span all phases of the cycle—and then stop injections and cause practically all ewes to ovulate and exhibit oestrus within 2-4 days, without depressing fertility. A significantly more uniform response is obtained if a stimulator of ovulation (PMS) is injected 24 hr after removing the inhibitor (progesterone).

Not one of 224 ewes treated came into oestrus until after injections ceased, when 211 were served within 4 days. Thus 13 ewes (5.8 per cent.) were not detected in oestrus after treatment. Of these, six were served by teasers one cycle later, suggesting the possibility of a "silent" oestrus. This record is better than that reported by O'Mary, Pope, and Casida (1950), who observed four of their 20 ewes, which also received 10 mg progesterone daily, to be served during the period of treatment. Hunter (1954), who treated 45 ewes in a similar manner, found that none came into oestrus during the period of injections, but seven (15.5 per cent.) failed to exhibit oestrus after cessation of treatment.

The percentage conception, as indicated by returns to service, was 60 per cent. as compared with 54.6 per cent. of ewes lambing. The discrepancy of 5.4 per cent. is composed of error due to inaccuracies in observing returns, to "silent" heats, and to loss of pregnancies after the first 3 weeks of gestation, and was distributed at random between treatments and sires. This suggests an extremely small loss of pregnancies after the stage of attachment, as has been previously affirmed by the author (Robinson 1951), and does not confirm the conclusion of Morley (1954) that such losses are likely to be large in Merino sheep.

Of particular significance is the interaction between the time of insemination, relative to cessation of progesterone treatment, and the lambing rate. When ewes come into oestrus and are inseminated within 72 hr of the final injection of progesterone—whether injected with PMS or not—the proportion which lamb is significantly greater than in ewes which are teased in the ensuing 24 hr and inseminated on the fourth day. Clearly, the time of ovulation, relative to insemination, in ewes inseminated on the third day more closely approaches the optimum, and this is independent of whether the ewes are first served early or late in the preceding 24 hr. The majority of ewes which have not been served within

72 hr—particularly if they have received PMS—are served within 84 hr; that is, they are in pro-oestrus within 72 hr. It appears probable that there is less variation in the time of ovulation, particularly in PMS-treated animals, than in the time of onset of oestrus. The time of ovulation following the injection of PMS to anoestrous sheep primed with progesterone is remarkably uniform (Robinson 1954, 1955) and the same is likely to be true in the breeding season when similar treatment is given. Hence it appears probable that the most satisfactory results would be obtained if progesterone-PMS treated ewes were inseminated on a time basis, regardless of the evidence of oestrus by the teasers. The same may not be true of animals treated only with progesterone, in which 8 per cent. were not served till 84-96 hr after the final injection.

It is apparent that oestrus and ovulation can be controlled in the Merino sheep in the breeding season, without impairment of fertility. A reasonable degree of control is attained with daily injections of 10 mg progesterone for the duration of one normal cycle, but this is significantly improved by the additional injection of 500 i.u. PMS one day later. This latter treatment results in such a precise oestrus-ovulation response that ewes can be inseminated 48 hr after the injection of PMS without reference to the results of teasing, with the prospect of 50-60 per cent. of ewes lambing and the likelihood of a high proportion of twin births. This permits careful planning of the whole operation, with maximum use of the semen of each ram on each day, the joining of any group of ewes to any ram desired on the day desired, and the concentration of insemination and of lambing into an extremely short span of time.

The widespread use of such a technique depends on its expense and the trouble involved relative to the gains obtained. A ram valued at a thousand guineas is likely to get perhaps 250 lambs in his active life—a service cost of four guineas. Hence the maximum use of such a ram, if a proven sire, is worth some considerable effort and expense. The materials used in this experiment are costly if bought in the present-day commercial preparations, but could be marketed for large-scale veterinary use at quite reasonable prices. The technique, as used, was tedious and time-consuming, but once organized was easily carried out. The intramuscular injection of large numbers of sheep can be done quickly, and the whole operation involved no more work than is needed for uncontrolled artificial insemination in which a large mob of sheep is mustered daily for several weeks and examined for teased ewes. Furthermore there is good reason to believe that the technique can be considerably simplified by practices such as the use of more slowly absorbed preparations of progesterone. Also the promising lambing results obtained using 0.05 ml of semen gives considerable hope of spreading the semen much further than was done in this experiment.

It is concluded that the use of progesterone, particularly in conjunction with PMS, is likely to be of practical value in sheep breeding. Its use, at least in the near future, is likely to be confined to the maximum use, by artificial insemination, of particularly valuable proven sires in studs where such use offsets the trouble and expense involved. The experiment reported here was a first attempt and gave most encouraging results. Further studies are needed on the time of ovulation relative to the injection of PMS, on optimum times of insemination, on the optimum dose of semen, and on the use of diluted semen. There is a strong possibility that the number of ewes inseminated in a limited period of time, and the lambing rate, can be considerably increased in the light of such additional knowledge.

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TOXAPHENE-RESISTANT CATTLE TICKS (*BOOPHILUS MICROPLUS* (CANESTRINI)) OCCURRING IN QUEENSLAND

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Summary

Cattle ticks on four herds in southern Queensland survived dipping in a vat charged with 0.5 per cent. w/v toxaphene, which nevertheless disinfested a fifth herd. Protective periods afforded by toxaphene against attachment of resistant larvae were much shorter than against susceptible strains. Laboratory concentration-response tests on a culture of the toxaphene-resistant ticks indicated that the median lethal concentration of toxaphene for engorged females was 19 times higher than that of a reference strain, and that for the larvae was also significantly higher.

The toxaphene-resistant ticks were not affected when cows were sprayed with 0.05 per cent. w/v gamma-BHC. This was consistent with an earlier history of BHC resistance in the ticks on these herds. Spraying trials indicated no marked arsenic resistance in the toxaphene-resistant ticks. The mortality of the toxaphene-resistant ticks on cattle sprayed with 0.5 per cent. w/v *pp'*-DDT, and the protective period against larval reinfestation, were as high as usually observed in other tick populations. A very high kill resulted from spraying the toxaphene-resistant ticks with 0.05 per cent. w/v diazinon.

Subcutaneous injections of peanut oil solutions of lindane, dieldrin, and aldrin at the rate of 25 mg toxicant/kg host body weight were without effect on toxaphene-resistant ticks.

I. Introduction

It has frequently been observed that selection of a resistant strain of an organism by exposure to one toxicant increases its resistance to others (e.g. see Babers and Pratt 1951). This tendency is exhibited by cattle ticks, since Fiedler (1952) and Whitnall *et al.* (1952) demonstrated that BHC-resistant *Boophilus decoloratus* (Koch) in South Africa were also resistant to toxaphene, and Freire (1953) found that the same held true for BHC-resistant *B. microplus* (Canestrini) in Brazil. Hitchcock (1953) found in laboratory trials that engorged females of BHC-resistant *B. microplus* from Queensland were resistant also to toxaphene. Field evidence of this phenomenon in Australia was, however, very limited (Anon. 1954) until a dip was charged in southern Queensland to study a toxaphene formulation. Here it proved impossible to control the ticks with toxaphene, apparently owing to the fact that they had previously developed a resistance to BHC. Because of this trouble the original purpose of the experiment could not be realized; but in view of the economic importance of acaricide resistance, and the disputes which usually surround

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early claims of the existence of resistant ticks, the observations made during the brief period for which the dip was available are recorded in the present paper.

II. Methods

In early September 1954 a concrete dip on a property near Beaudesert, south Queensland, was thoroughly cleaned and charged with 0.5 per cent. w/v toxaphene as an emulsion. As the dipping of cattle lowered the level of the liquid, this was restored periodically during the trials by the addition of rain-water from a tank, and extra toxaphene concentrate in the exact amounts required for the added water.

Four herds from adjoining properties were dipped in this vat, and also occasional mobs of tick-infested cattle from a property some miles distant. The cattle ranged in age from calves to aged cows, and were of various breeds and grades.

Detailed examinations of tick infestations on the flanks, escutcheons, and necks of some animals were made in the crush leading to the dip. Separate records were made of the abundance of all the tick life-history stages recognized by Hitchcock (1955). General examination of larger numbers of animals in the yards and draining pen permitted rough comparisons of the infestations of the more advanced stages of ticks.

Observations on dipped cattle were supplemented by knapsack-spraying experiments with toxaphene and other acaricides. There is always an element of doubt about thoroughness of coverage on hand-sprayed animals, and it is suspected that there are other differences between the two techniques: for instance, it is only on the day of charging that the liquid in the vat is strictly comparable with the freshly prepared material used in sprayers. Hence a distinction has been made between dipping and spraying experiments in the presentation of results.

Cultures of toxaphene-resistant ticks for laboratory studies were reared on animals maintained in isolation at Yeerongpilly. The Yeerongpilly strain was used for comparison. This had been maintained in pure culture without acaricidal treatment for over 8 years, and had never been exposed to chlorinated acaricides.

Laboratory dosage-mortality trials with engorged adults and larval ticks were performed by modifications of the methods outlined by Hitchcock (1953). The tests with adult ticks were, in fact, performed in substantially the same manner; but some effort was made to standardize drainage from all ticks by contact with filter paper after immersion in the test fluids. More marked departures from Hitchcock's techniques were made in the trials with larvae. After the specified period of immersion, the test liquid containing the larvae was emptied into a small funnel having a partition of 60 mesh bronze gauze set into the top of the stem. The liquid was removed from the larvae as far as possible by suction, after which they were blown with compressed air off the gauze into a

ventilated tube for observation of mortality. It is considered likely that irregularity of residual deposit interferes with the reproducibility of results in trials with larvae to a much greater extent than in trials with engorged females. Hence in some tests the residual toxaphene was washed from the larvae with 0.05 per cent. w/v "Triton X100" wetting agent while they were still on the filter.

III. Results

(*a*) *Response of Ticks to Toxaphene*

(i) *Dipped Cattle.* (1) *Toxaphene-resistant Ticks.*—The most detailed observations were made on the cattle of herd A. The records of the tick infestations on animals from this herd are summarized in Table 1. When these cattle were first dipped in toxaphene on September 7, 1954, they were lightly infested with ticks. A high proportion of nymphs and adult ticks survived this dipping. Larvae were not detected 6 and 13 days after dipping. This, and the representation of life-history stages at 27 days, suggest that there may have been a protective period (Norris, Roulston, and Snowball 1950) of more than a week following the first dipping; but the assessment of persistency is unreliable when larvae are as scarce as they were even on untreated cattle at this time.

Larval attachment may also have been prevented for about 6 days following the second dipping, which took place 27 days after the first. Many nymphs and adult ticks also survived this dipping.

The kill of ticks became progressively poorer, and by January 1955 the ticks appeared to be quite unaffected by the dip. After the second dipping, moreover, there was clearly no protective period. Observations were not possible after January because the toxicant had to be changed to bring the ticks under control.

Cattle from three neighbouring properties were also dipped in this vat. Isolated observations made on herds B and C and reports from the graziers indicated that the toxaphene at no time effected a complete kill of ticks on these herds. The fourth herd, D, was not studied; but reports from the graziers indicated well-marked toxaphene resistance. For instance, the herd was dipped on January 2, 1955, without apparent effect on the heavy tick infestation present. The cattle were redipped on January 10-11, again without apparent effect. Engorged ticks picked from a bull the day following the second dipping were able to crawl and appeared normal in spite of the two dippings in the course of their parasitic life cycle. The history of the tick infestation on herds B, C, and D in fact paralleled that of herd A closely.

Analyses of Dipping Fluid.—The results of analyses of fluid from the Beaudesert dip are presented in Table 2. The samples taken on January 27, 1955, and February 4, 1955, indicate that the toxaphene tends to settle

to the bottom of the vat. The bearing of this and the progressive decrease in the concentration of the stirred dip on the tick mortalities observed are discussed in Section IV.

TABLE 1

DATES OF DIPPINGS OF HERD A IN TOXAPHENE AND INFESTATIONS OF *BOOPHILUS MICROPLUS* OBSERVED ON CATTLE

Date Dipped	Days Since Previous Dipping	Number of Cattle Examined	Tick Infestation on Cattle
7.ix.1954		4	Lightly infested with all stages*
	6	5	Lightly infested with engorged nymphs and all adult stages
	13	6	Lightly infested with males, medium-sized, and older females
4.x.1954	27	5	Lightly infested with all stages except semi-engorged and engorged females*
	7	4	Lightly infested with seed ticks, engorged nymphs, and all adult stages
	21	2	Lightly infested with all stages
8.xi.1954	34	4	Lightly to heavily infested with all stages*
	8	9	Lightly to heavily infested with all stages except medium-sized and semi-engorged nymphs
23.xi.1954	15	12	Moderately to heavily infested with larvae, lightly to moderately with all other stages*
	7	3	Lightly infested with all stages
	14	6	Lightly to heavily infested with all stages
	21	3	Lightly to heavily infested with all stages
21.xii.1954	28		Not examined
7.i.1955	17		Not examined, but reported heavily infested*
	7	10	Lightly to heavily infested with all stages
27.i.1955	20	13	Lightly to heavily infested with all stages
	5	2	Lightly to moderately infested with all stages*

* Infestation present immediately before the dipping on date in first column.

(2) *Toxaphene-susceptible Ticks.*—Sometimes mobs of tick-infested cattle were brought to the toxaphene vat from a property 12 miles distant (herd E). Cattle on this property had been dipped exclusively in arsenic until 1953, when a small quantity of DDT emulsion was added to the dip. The cattle from herd E were usually dipped in toxaphene within a day or so of arrival, and this invariably produced a complete kill of their ticks. For instance, one tick-infested mob from herd E was dipped in the

toxaphene vat on September 25, 1954. The rapid destruction of the ticks was reported. Several beasts examined 9 days later were quite free of surviving ticks, and six head examined 16 days after dipping were reinfested with ticks up to the young nymph stage, but quite free of older ticks.

Further tick-infested cattle from herd E were dipped on November 20, 1954. Three head, known to have been heavily infested before dipping, examined 3 days after dipping were completely free of live ticks. At this time some ticks of all stages survived dippings on the local herds, A-D.

TABLE 2
ANALYSES* OF SAMPLES FROM TOXAPHENE-CHARGED VAT

Date of Sampling	Nature of Samples	Toxaphene Concn.† in Duplicate Samples
7.ix.1954	Surface of newly charged vat after dipping 23 head	0.560 0.530
25.x.1954	Surface after dipping 72 head	0.388 0.397
27.i.1955	Unstirred surface	0.044 0.044
	Surface after dipping 72 head	0.257 0.258
4.ii.1955	Liquid sludge after emptying vat	0.978 1.030
	Hard bottom deposit under sludge	1.680 1.530

* Toxaphene estimated by method described in Hercules Powder Company's "Toxaphene Manual" (1948).

† Per cent. w/v except for samples collected 4.ii.1955, which are expressed as per cent. w/w.

Although the ticks present on the cattle from herd E when they were brought in were all killed by dipping in the toxaphene vat, once the cattle were released in the pastures in the vicinity of the dip they were exposed to reinfestation by toxaphene-resistant larvae, and the protective periods would have been similar to those observed on the local herds. No opportunity arose for the assessment of the protective period afforded by the Beaudesert dip against toxaphene-susceptible ticks; but observations made at Ormeau, about 25 miles from Beaudesert, are presented for comparison.

In June 1954, 34 dairy cattle were dipped in the Ormeau vat, which had been charged with 0.5 per cent. w/v toxaphene as an emulsion about 5 months before. Before dipping, the cattle were infested with all stages of ticks, and semi-engorged and engorged females were estimated to number at least 300 per beast. Only a few engorged nymphs and a half-dozen engorged adult ticks were found on the entire herd 3 days after dipping. The nymphs detected at 3 days were probably destroyed by

residual toxaphene after moulting and no ticks were found 10 days after dipping. The cattle still appeared to be completely tick-free at 17 days. Thirty days after dipping, some reinfesting ticks had attained the medium-sized nymph stage, while 44 days after dipping, the oldest ticks present were medium-sized adults, a stage attained on the average about 19 days after their attachment as larvae (Hitchcock 1955). Arsenic-dipped cattle grazing in the same pastures were heavily infested with larvae throughout the period of observation, and without question the toxaphene-dipped cattle were protected from invading seed ticks for at least 3 weeks after dipping. This protective period is longer than that found by most other workers, but Laake (1949) found that 0.5 per cent. w/v toxaphene protected cattle for up to 4 weeks against infestation by *B. microplus* in Brazil.

The above description of the results at Ormeau does not apply to one cow which was known to be the first of the herd through the unstirred vat. The kill of ticks on this cow was incomplete, and the protective period nearly a week shorter than on the rest of the herd. This cow probably received inadequate treatment because of the tendency for the toxaphene to settle to the bottom of the vat between dippings.

The cattle in the Ormeau herd had previously been dipped exclusively in arsenic, and hence the ticks had never been exposed to chlorinated acaricides.

(ii) *Sprayed Cattle.* (1) *Toxaphene-resistant Ticks.*—By January 1955 it was apparent that the Beaudesert dip was even less efficient in controlling the ticks than when charged in September 1954. Spraying trials were carried out to determine whether this could have been due partly to the decrease in toxaphene concentration indicated in Table 2.

On January 21, 1955, four moderately to heavily infested cows from herd B were sprayed with freshly prepared 0.5 per cent. w/v toxaphene of the same formulation as in the Beaudesert vat, using at least 1½ gal per beast. Six hours after spraying, considerable numbers of adult ticks had engorged on all cows. Some males and a few medium-sized adult females were dead. Three days after spraying, large numbers of engorged nymphs and recently moulted females remained; but there had been a rather high mortality of all other stages in comparison with contemporary dipping. Six days after spraying, the surviving ticks on the cows still totalled many hundreds. Eleven days after spraying, survivors comprised a few males and semi-engorged females estimated to number 5, 50, 50, and 300 respectively on the four beasts, the numbers being roughly proportional to the degree of infestation of the cows before spraying. Some reinfesting ticks had attained the medium-sized nymph stage on three of the beasts, indicating a protective period of no more than 2 days.

The mortality in the above spraying trial was considerably higher than in contemporary dippings of the same herd in the vat, but was still inadequate for practical tick control.

Three moderately infested cows from herd A were similarly sprayed with 0.5 per cent. w/v toxaphene on January 27, 1955. Five days after spraying, a high proportion of medium-sized nymphs and older stages survived. Larvae were much fewer than before spraying, but only undistended nymphs appeared to be completely lacking from all beasts. Eleven days after spraying, all stages of larvae and nymphs were abundant on the cows, which were estimated to carry 50, 120, and 220 semi-engorged females respectively. Undistended, medium-sized, and engorged females were, however, comparatively scarce.

The kill of ticks in this trial was higher than observed in contemporary dippings of the same herd in the vat, but decidedly lower than in the above spraying trial with cattle from herd B. The protective period was negligible.

TABLE 3

PERCENTAGES OF ENGORGED FEMALES OF BEAUDESERT AND YEERONGPILLY STRAINS OF *BOOPHILUS MICROPLUS* LAYING NO VIABLE EGGS AFTER DIPPING IN VARIOUS CONCENTRATIONS OF TOXAPHENE

All percentages based on at least 100 ticks

Beaudesert Strain		Yeerongpilly Strain	
Toxaphene Concentration (% w/v)	Engorged Females Laying No Viable Eggs (%)	Toxaphene Concentration (% w/v)	Engorged Females Laying No Viable Eggs (%)
0.5	10	0.016	2
1.0	38	0.031	8
2.0	75	0.062	45
4.0	93	0.125	84
8.0	91	0.25	93
10.0	100	0.50	100

(2) *Toxaphene-susceptible Ticks.*—By contrast with the Beaudesert results cattle sprayed with 0.5 per cent. w/v toxaphene at Caboolture, Qld. (Commonwealth Scientific and Industrial Research Organization 1953) were completely freed of ticks and there was a protective period against reinfestation (of about 12 days; R. A. J. Meyers, unpublished data).

(iii) *Concentration-response Tests with Toxaphene against Resistant and Susceptible Ticks.*—A few engorged female ticks collected from cattle of herd A on January 14, 1955, laid large batches of fertile eggs when kept in an incubator at 30°C and high humidity. The larvae were reared to maturity in isolation on a bovine. To obtain sufficient material for laboratory trials the progeny of this culture were similarly reared.

Engorged females from the second culture and engorged females of the Yeerongpilly strain were immersed in various concentrations of

toxaphene, using the same formulation as in the Beaudesert dip. The percentages of females of both strains not laying viable eggs* are illustrated in Table 3. Probit analysis of the concentration-response data was carried out by the method of Finney (1947). It was determined that the concentration of toxaphene required to prevent 50 per cent. of engorged Beaudesert ticks from laying viable eggs was 1.36 per cent. w/v (1.06-1.77).† The corresponding concentration for engorged females of the Yeerongpilly strain was 0.07 per cent. w/v (0.05-0.09). The relative toxaphene resistance of the Beaudesert ticks was therefore 19.2 (13.1-27.7) times that of engorged females of the Yeerongpilly strain.

TABLE 4

PERCENTAGE MORTALITY OF LARVAE OF BEAUDESERT AND YEERONGPILLY STRAINS OF *BOOPHILUS MICROPLUS* 24 HR AFTER DIPPING IN VARIOUS CONCENTRATIONS OF TOXAPHENE AND WASHING WITH DILUTE WETTING AGENT

Each percentage the mean of three tests each with over 100 larvae

Beaudesert Strain		Yeerongpilly Strain	
Toxaphene Concentration (% w/v)	Larvae Dead 24 Hr after Immersion (%)	Toxaphene Concentration (% w/v)	Larvae Dead 24 Hr after Immersion (%)
		0.32	24
0.64	8	0.64	46
1.28	14	1.28	63
2.56	14	2.56	87
5.12	27	5.12	90
10.24	46		

Concentration-response tests were also carried out with larval progeny of both the above cultures using the technique of washing away residual toxaphene with a dilute wetting agent. The mortalities recorded 24 hr after immersion are presented in Table 4. The median lethal concentration of toxaphene for the resistant strain was 17.02 per cent. w/v,‡ whilst the corresponding concentration for the Yeerongpilly strain was 0.77 (0.59-1.09). The lines obtained by plotting probit mortality against the logarithm of the concentration of toxaphene for the larvae of the two strains were, however, not parallel. The median lethal concentration for the Beaudesert larvae was 22.1 (7.2-68.4) times higher than that for the Yeerongpilly strain, and at 25 per cent. lethal concentration the ratio was 12.4 (6.1-25.2).

Washing the residual toxaphene from the larvae soon after immersion as in the above experiments has the effect of raising the median lethal concentrations and increasing the difference in degree of resistance

* The accepted criterion of mortality in tests with engorged females.

† Fiducial limits at the 5 per cent. level are indicated by brackets in this section.

‡ The lower fiducial limit to the median lethal concentration is 8.2. The upper limit is fictitious in that it corresponds to a concentration in excess of 100 per cent.

between the two strains. It will be noted, in fact, that the median lethal concentrations for larvae of the two strains are much higher than the corresponding values for engorged females of the same strain. This is contrary to the general experience that larvae are far more likely to be destroyed by a particular concentration of toxicant than are engorged adults. The higher median lethal concentration for larvae in the above tests must, in fact, have been due largely to the washing technique employed in the larval trials, and possibly also to the fact that tests with larvae terminate after 24 hr, whereas the toxicant is allowed to exert its maximum effect on engorged females before results are assessed.

TABLE 5

PERCENTAGE MORTALITY OF LARVAE OF BEAUDESERT AND YEERONGPILLY STRAINS OF *BOOPHILUS MICROPLUS* 24 HR AFTER DIPPING IN VARIOUS CONCENTRATIONS OF TOXAPHENE AND ALLOWING RESIDUAL DEPOSIT TO REMAIN ON LARVAE

Each percentage the mean of four tests each with over 100 larvae

Beaudesert Strain		Yeerongpilly Strain	
Toxaphene Concentration (% w/v)	Larvae Dead 24 Hr after Immersion (%)	Toxaphene Concentration (% w/v)	Larvae Dead 24 Hr after Immersion (%)
0.045	22	0.0056	15
0.09	30	0.011	41
0.18	40	0.022	32
0.36	59	0.045	54

The following data illustrate the effect of not washing residual toxaphene from larvae after immersion. Ticks gathered from herd A on February 7, 1955, laid large numbers of fertile eggs, and the resulting larvae were used to infest a bovine. Larval progeny from this culture were used in concentration-response tests without washing off the residual toxaphene. The percentage mortalities are presented in Table 5. The median lethal concentration of toxaphene for the Beaudesert larvae was 0.27 per cent. w/v (0.15-0.89), while that for the Yeerongpilly larvae was 0.04 per cent. w/v (0.02-0.14). In this case the Beaudesert ticks exhibited 6.9 (2.4-19.0) times the toxaphene resistance of the Yeerongpilly strain at the median lethal concentrations. The median lethal concentrations in this test are considered to bear a more realistic relation to those of the engorged females of the corresponding strain than those in the trials with washed larvae.

(*b*) *Response of Toxaphene-resistant Ticks to Acaricides other than Toxaphene*

(i) *Spraying Trials.* (1) *BHC.*—Three lightly to heavily infested cows from herd A were treated by knapsack sprayer with 0.05 per cent.

w/v gamma-BHC prepared from a commercial paste on January 27, 1955. Five days after spraying, almost all ticks on the cattle appeared to be unaffected. Eleven days after spraying, certain of the life-history stages could not be detected on one of the cows, which, however, was very lightly infested at the time of spraying, and may have been somewhat tick-resistant. The ticks on the other two cows still appeared to be as numerous as before spraying. On the 11th day the three cows were estimated to carry respectively 30, 125, and 160 semi-engorged adult ticks. By contrast, BHC-susceptible ticks (Norris, Roulston, and Snowball 1950) are almost all strongly affected within a few hours, and nearly complete mortalities result from dipping in freshly-prepared 0.05 per cent. w/v gamma-BHC wash. It is evident that the ticks on herd A were highly BHC-resistant.

(2) *DDT.*—Three lightly to heavily infested cows from herd A were treated by a knapsack sprayer with 0.5 per cent. w/v *pp'*-DDT as a colloidal preparation on January 27, 1955. Five days after spraying, the cows carried only a few engorged nymphs and newly moulted adults, and a small number of semi-engorged females, examples of which, when removed from the beasts, exhibited the incoordination characteristic of DDT poisoning. Eleven days after spraying, the cows had a few surviving males and respectively 5, 50, and 55 semi-engorged female ticks which had survived the spraying as engorged nymphs. Larvae were also present on each cow, some having attained full engorgement, indicating a protective period of approximately a week. This result with DDT approximates closely to that usual in other tick populations, and there is no suggestion of DDT resistance. This was confirmed in February, when, after the removal of the toxaphene, the vat was charged with the same colloidal DDT preparation as used in the spray trials. Excellent control of ticks then resulted.

(3) *Arsenic.*—Three moderately tick-infested cows from herd A were sprayed with a commercial sodium arsenite preparation containing 0.1 per cent. w/v arsenious oxide on January 27, 1955. By the fifth day after spraying, there was little effect on the infestation except a slight discoloration of some of the engorged adults. As other suitably infested cows were unprocurable, the same three animals were sprayed with a preparation containing 0.2 per cent. w/v arsenious oxide as originally intended, on the fifth day after the first spraying. Six days after the second spraying there had been a noticeable reduction in the infestations and almost all semi-engorged and engorged female ticks, estimated to number 7, 50, and 110 respectively on the three cows, exhibited characteristic symptoms of arsenical poisoning. In spite of the double spraying it seems justifiable to conclude that the ticks on herd A exhibited at most arsenic resistance of a low order, when compared with well-marked cases such as that cited by Legg and Shanahan (1954), in which ticks were able

to develop normally on cattle in spite of 10 dippings in a dip containing 0.2 per cent. w/v arsenious oxide at intervals of 5-8 days.

(4) *Diazinon.*—This was selected as a representative of the group of organic phosphate acaricides which were known to show promise for the control of ticks resistant to chlorinated acaricides (Commonwealth Scientific and Industrial Research Organization 1955). Four moderately to heavily infested cows from herd B were sprayed with 0.05 per cent. w/v diazinon on January 24, 1955. It was reported that by the following day the cows appeared to be almost free of live ticks. Three days after spraying, very few nymphs and a total of only two strongly affected semi-engorged adults survived on the four cows. Six days after spraying, extensive search of the escutcheons, flanks, and necks of all four cows revealed a total of about 40 engorged nymphs and about six undistended and medium-sized adults. Eleven days after spraying, a few males survived on the diazinon-sprayed cows, which also bore respectively 0, 10, 20, and 28 semi-engorged adult ticks. By the 11th day after spraying, some reinfesting ticks had attained the medium-sized nymph stage, indicating a protective period of only about 2 days. Complete kills and a protective period of "not more than 4 days" have been found in power-spraying susceptible ticks with 0.05 per cent. w/v diazinon at Amberley, Qld. (Commonwealth Scientific and Industrial Research Organization 1955); but the slightly inferior results at Beaudesert are considered to reflect differences in thoroughness of spraying rather than differences in susceptibility to diazinon.

(ii) *Subcutaneous Injections with Lindane, Dieldrin, and Aldrin.*—On November 30, 1954, three cows from herd A were injected respectively with peanut oil solutions of lindane, dieldrin, or aldrin at the rate of 25 mg toxicant/kg body weight as described by Roulston (unpublished data). The weights of the animals were estimated by two experienced cattlemen.

Examinations 7, 14, and 21 days after injection revealed no effect on the tick population. Had the ticks been susceptible to these toxicants (Roulston, unpublished data) larval reinfestation would have been interrupted on the lindane-injected animal from about the seventh until at least the 14th day, and on the dieldrin-injected animal from about the 14th day onwards, and there would have been an extremely light infestation of larvae on the aldrin-injected animal by the 21st day. There would also have been some destruction of the younger life-history stages present at the time of injection, whereas no tick appeared to be affected.

These results confirmed the BHC resistance of the toxaphene-resistant ticks, and suggested that they were resistant to dieldrin and aldrin.

Subcutaneous injections with toxaphene, DDT, and diazinon were not attempted, as these acaricides appear to exert no systemic effects even on susceptible ticks (Roulston, unpublished data).

IV. Discussion

The results presented in this paper amply demonstrate that the ticks on the Beaudesert properties were resistant to toxaphene, but indicate a situation of some complexity. The kill of ticks undoubtedly decreased as the dipping fluid aged. Spraying trials indicated that this was at least partly due to deterioration in the dipping fluid; but it is not possible to say whether there was a parallel increase in the degree of resistance of the ticks. This could have been established if concentration-response trials had also been carried out with cultures of ticks established early in the course of the experiment. Increase in the degree of resistance of organisms on continued exposure to toxic substances is a familiar phenomenon; but usually many generations elapse before the increase becomes marked. The Beaudesert experiment, however, ran for less than 5 months, which is sufficient for less than three generations of cattle ticks and would not afford much opportunity for increase in the resistance of a genetically homogeneous strain. However, it is extremely doubtful whether the ticks on the properties were of homogeneous stock. There was considerable movement of cattle from other herds to and from the dip through the pastures of herd A, purchases of infested cattle were sometimes made, and stock newly transferred from herd E (non-resistant ticks) and other places were sometimes allowed to remain in the pastures for several days before dipping. During such periods introduced cattle probably dropped considerable numbers of toxaphene-susceptible ticks. It is almost certain, therefore, that seed ticks in the pastures at the inception of toxaphene dipping were of mixed origin. However, susceptible ticks would have been eliminated in the earliest dippings in toxaphene, resulting in higher kills in the initial stages. As the experiment progressed, the progeny of the toxaphene-resistant survivors would have comprised an increasing percentage of the tick population. Thus, although the increase in maximum degree of resistance of ticks on the cattle throughout the experiment may not have been great, the ticks would have been more uniformly resistant by the end of the experiment. Progressively poorer kills may therefore have resulted, even if the condition of the dip fluid had remained unchanged.

The degree of toxaphene resistance of the Beaudesert ticks relative to the Yeerongpilly strain as determined from laboratory trials is necessarily somewhat arbitrary. Not only is it affected by the progressive selection against susceptible individuals, discussed above, in relation to the time of establishment of the culture, but the susceptibility of the reference strain could easily differ from that of other susceptible strains. An indication that this was so for two strains of arsenic-susceptible ticks was given by Hitchcock and Roulston (1955). The applicability of the principle to other toxicants may reasonably be presumed.

The technique of the laboratory trials fails to reproduce practical dipping conditions, in that the ticks are not in continuing contact with

toxaphene-impregnated hair, while the results of the two types of trials with larvae indicate the extent to which differences in technique may influence the median lethal concentration determined, and the ratio of the median lethal concentration of the resistant to that of the reference strain. Though artificial from these aspects, the laboratory trials afford quantitative evidence of resistance of a type which it is impossible to obtain in field trials.

The loss in toxicity of the fluid in the vat in the course of the experiment is probably attributable to decrease in toxaphene concentration rather than to a loss in relative toxicity such as occurs in some BHC vats (e.g. Roulston and Hitchcock 1953). Sparr *et al.* (1952) found that the amounts of toxaphene carried out of dips on the coats of cattle actually increased as the emulsions aged, owing to increase in the droplet size of the disperse phase. Presumably a similar phenomenon occurred in the vat under discussion, but this effect was outweighed by the reduction in gross toxaphene content of the dipping fluid.

Probably the decrease in concentration of the fluid is partly attributable to the stripping effect described above, and partly to the imprisonment of toxaphene in the consolidated bottom deposit (Table 2). There was also an increase in the inorganic chloride content of the vat from 0.3 mg/g water at the time of charging to 1.07 by January 27, 1955. This may indicate some decomposition of toxaphene; but could also be accounted for by contamination by urine, and progressive removal of chlorides from the coats of dipped cattle. Whatever the causes of the decrease in concentration, there is no suggestion that the chemical history of the dip was other than normal for such toxaphene emulsions, nor that the formulation in question is not of great value for the control of most tick populations. It may be presumed that in the control of susceptible ticks a decrease in toxaphene concentration of the order observed is of little consequence. DDT and BHC vats also are known to undergo progressive decrease in the concentration of the active constituents until a more or less stable working level is attained (Roulston, unpublished data).

The tendency for the toxaphene to settle to the bottom of the vat may have led on occasions to the inefficient dipping of individual animals in the herds. However, it is improbable that this had much effect on the data included in Table 1, and general inspection of the herds usually indicated that the samples examined in detail were fairly representative.

The history of tick control on the four Beaudesert properties is relevant to the discussion of toxaphene resistance. Arsenic had commenced to give unsatisfactory results in 1949, when as many as three dippings in 30 days in dips containing 10 lb arsenious oxide/400 gal did not free the cattle of ticks. The vat was charged with BHC early in 1950, and gave satisfactory results for about 18 months, when a period of poor control ensued. Results were not improved when, after 2 years, the vat

was cleaned out and recharged with fresh BHC, and used for a further year. The cattle were never free of ticks, though dipped at 3-weekly intervals. A DDT emulsion had given satisfactory results for 12 months prior to charging the vat with toxaphene. There is little question that the BHC resistance which the ticks had previously developed conferred on them their resistance to toxaphene.

The systemic injections suggested that the Beaudesert ticks were resistant to dieldrin and aldrin, in addition to gamma-BHC and toxaphene. Tests reported with BHC-resistant ticks in north Queensland (Anon. 1954) suggested that these also were resistant to toxaphene and, in addition, to chlordane and heptachlor; but like the Beaudesert ticks, were susceptible to DDT. It seems likely that the pattern of resistances in houseflies described by Busvine (1954) is closely paralleled in cattle ticks, and presumably one could also apply this author's theory that the linkage is due to similarity in molecular configuration between the substances to which resistance is exhibited, in contrast with an entirely different molecular pattern in DDT.

The spraying trials with toxaphene suggested that the ticks on two adjoining properties had different degrees of resistance to toxaphene. This was indicated by visual estimation of the proportion killed, the difference in the numbers of life-history stages surviving spraying on each group, and the difference in protective periods. Although there would certainly be some exchange of tick population between the two places because of the use of a common dip, the development of different degrees of resistance is not unlikely. By January 1955, herd B had been dipped four times in toxaphene, as against six dippings of herd A. The owner of herd B was also apt to select only heavily infested animals for dipping on some occasions. If these differences in dipping practice were of long standing, there might have been less intensive selection for BHC resistance in earlier years among the ticks on herd B, and hence differing degrees of BHC resistance on the two properties when the use of toxaphene commenced. On the other hand the differences in husbandry methods could with equal justification be supposed to have produced a difference in the degree of uniformity of the toxaphene resistance of the ticks on the two properties. Non-resistant diluents of the tick population would have had a greater chance of survival under the system of management of herd B. Thus the ticks which survived spraying on herd A may not have had a higher degree of toxaphene resistance than the survivors on herd B, but because they constituted a greater proportion of the tick population, lower kills resulted. Once again, such problems could best be resolved by laboratory studies of cultures from both herds, but facilities severely limit this aspect of the investigations.

Reports of the experience of several other cattle owners near Beaudesert suggest that BHC-toxaphene-resistant ticks are not confined

in that district to the four properties mentioned in the present paper. They have been reported from north Queensland (Anon. 1954), and their occurrence at Rockhampton in central Queensland was also demonstrated in January 1954 by Mr. P. R. Wilkinson (unpublished data) in the course of small-scale spraying trials. In view of the widespread replacement of BHC in vats to surmount the problem of resistance, and the fact that toxaphene resistance has also been known to develop in *B. microplus* not previously exposed to BHC (Freire 1953), reports of other cases in Queensland may be expected.

The practical importance of these observations and those of overseas workers is that toxaphene is not to be recommended as an alternative acaricide where ticks have developed BHC resistance. This statement applies particularly to Australian conditions of management, as Whitnall *et al.* (1952) were satisfied with the control of BHC-resistant *B. decoloratus* achieved by weekly treatment with toxaphene, although their laboratory work demonstrated pronounced toxaphene resistance in the ticks. It is likely that dieldrin and aldrin are also unsuitable for the control of BHC-resistant cattle ticks, whereas DDT is the only organic acaricide that can, at the moment, be recommended with confidence for use in dips. It is questionable whether reversion to arsenic is justifiable. Whitnall and Bradford (1947) cited cases in which the intensity of arsenic resistance diminished when ticks were not exposed to dipping in arsenic for a period of less than a year. Possibly after the use of arsenic has been discontinued for a period because of resistance, the ticks in many cases would be found to be more susceptible; but probably the degree of arsenic resistance would increase rapidly once again on reversion to this acaricide. The high degree of susceptibility of the toxaphene-resistant ticks to diazinon is encouraging, but unfortunately, like related compounds, this substance is too unstable for use in dips and must be applied as a freshly prepared spray.

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THE EFFECT OF SELF-LICKING BY CATTLE ON INFESTATIONS OF CATTLE TICK, *BOOPHILUS MICROPLUS* (CANESTRINI)

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Summary

Infestations of *Boophilus microplus* (Canestrini) were established by applying single batches of larvae to individually stalled cattle, and the percentage yields of engorged female ticks from animals kept in harness to impede licking were compared with those from animals allowed to lick freely.

In the harness trials, 33 per cent. of female ticks survived to fall as engorged adults from the host; in individual infestations this survival ranged from 5 to 69 per cent. In the trials without harness the corresponding survival was 9 per cent., ranging from 0.1 to 32 per cent. Statistical analysis showed a highly significant effect of harness in promoting survival of ticks.

Licking (and other forms of host behaviour, e.g. kicking and rubbing, which produce tick mortality by mechanical means) must be considered in any study of natural mechanisms regulating cattle tick populations.

I. Introduction

Research carried out since 1941 by the Division of Entomology, C.S.I.R.O., on the cattle tick (*Boophilus microplus* (Canestrini)) necessitated the regular culturing of ticks on stalled cattle to provide engorged females for use in acaricidal and ecological studies. The number of ticks falling from the cattle were quite often inadequate for the requirements of the research programme. As shortage of stalls precluded the use of more cattle to guarantee an abundant supply of ticks, measures had to be considered to ensure the maximum possible yield from each beast infested. Norris (1947) considered that licking by cattle was one of the factors responsible for the removal of DDT deposited on their coats by spraying or dipping. Hackman (1947), on the basis of experiments with stalled cattle, attributed to licking a major role in the disappearance of DDT from cattle hair. Hence it seemed possible that licking might also cause mortality of ticks, and experiments were carried out at Yeerongpilly, Qld., between October 1949 and September 1950 to determine whether tick yields were improved if the cattle were prevented from licking.

II. Materials and Methods

The stalls in which the cattle were kept were roofed but open-sided, hence the beasts were protected from rain, and for most of the day from direct sunlight. Each stall was surrounded by a water-filled moat which prevented the escape of ticks falling from the host.

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Ten Illawarra Shorthorn steers were used. These ranged in age from 6 to 12 months when the observations commenced.

Throughout each infestation the steer was tethered to a rail of the stall. To initiate the infestation, a broad sheet of hessian was fastened securely around the thorax of the steer, and a small metal cylinder containing a weighed mass of tick larvae from a laboratory culture was placed inside the sheet and then opened. Within a few hours the larvae had left the cylinder and spread out over the body of the steer. The hessian sheet was kept around the steer for 4 days, and then removed. This allowed the larvae ample time to insert their mouthparts into the host, for Hitchcock (1955) estimated that 95 per cent. of tick larvae which attach do so within an hour, although a small proportion may wander in the hair for as long as 3 days. The number of larvae originally in the cylinder was determined by weighing and counting a smaller batch of larvae from the same laboratory culture.

The numbers of engorged female ticks which fell from the host each day were recorded. Falling began not earlier than 17 days and ceased not later than 30 days after the application of the larvae. The steer was kept in the stall until all female ticks surviving from the original batch had dropped, and was then turned out to graze in a tick-free paddock until required for a further infestation, so preventing overlapping of successive infestations on the same animal.

A special harness was used to hinder a steer from licking itself. This consisted of a piece of strong timber secured at one end to the headstall and at the other to a surcingle just behind the forelegs so that it passed between the forelegs (Hooker, Bishopp, and Wood 1912, Plate 1, Fig. 5). Harnessed cattle could sometimes reach part of the thorax with the tongue by throwing the head back vigorously but such licking was very limited and comparatively ineffective.

III. Results

In Table 1 the data for the various infestations are given. The percentages of female ticks surviving (last 2 columns), referred to hereafter as "survival percentages", were calculated as:

$(2 \times$ no. of engorged female ticks falling/no. of larval ticks applied$) \times 100$,

i.e. it was assumed that 50 per cent. of the larvae in each batch applied were males and 50 per cent. females, though no criterion for distinguishing the sexes in the larval stage was known.

The average survival percentages were: trials with harness (host-licking impeded), 33; trials without harness (licking not impeded), 9. The ranges of the survival percentages in individual infestations were: with harness, 5-69; without harness, 0.1-32.

The age of larvae at the time of infestation varied, as did the number of larvae applied.

The correlations of survival percentage with larval age and with number of larvae applied were calculated for the trials without harness and were not significant. Hence the survival percentages were not affected by these two variables, at least within the ranges used.

In assessing the effect of harness on survival percentage, it is important to remove the possible effect of a time trend on this comparison, because the average date of trials with harness is later than that of trials without harness. An analysis of regression slopes fitted to the observations for the trials without harness indicated a significant general increase with time, the variation in slopes for different animals not being significant.

The assumption of linearity can only be regarded as approximately representing the character of the trend, but the sparsity and general nature of the data do not warrant further refinement. There is a trend towards higher recoveries of female ticks over the period of the observations, the pooled regression slope being 1.00 per cent. of survival per month. This could be a real trend in animal reaction, or one induced by some unrecognized changes in diet or stall conditions.

TABLE 1

EFFECT OF SELF-LICKING BY CATTLE ON ARTIFICIAL INFESTATIONS OF CATTLE TICK

Beast No.	Date of Infestation	Max. Age of Larvae (days)	No. of Larvae Applied	No. of Engorged Female Ticks Falling	Female Ticks Surviving (%)	
					Host Restrained from Licking	Host not Restrained from Licking
1	3.x.1949	31	57,600	2,468	8.6	
	21.xi.1949	20	25,300	6,400	50.5	
	31.i.1950	9	42,000	333		1.6
	21.iii.1950	7	41,300	482		2.3
	23.vi.1950	7	42,100	1,101		5.2
2	7.xi.1949	7	30,600	1,117		7.3
	9.i.1950	12	46,500	12,935	55.7	
	7.iii.1950	11	55,600	926		3.3
	4.v.1950	10	55,700	1,406		5.0
	4.vii.1950	7	76,200	1,021		2.7
	29.viii.1950	7	55,700	10,241	36.8	
3	10.x.1949	7	32,400	5,001	30.9*	
	3.i.1950	8	35,300	1,535		8.7
	28.ii.1950	7	29,600	3,026		20.5
	1.viii.1950	8	45,900	6,715	29.2	
	12.ix.1950	7	67,900	10,071	29.6	
4	21.xi.1949	20	50,000	6,400	25.6	
	7.ii.1950	24	42,300	3,341		15.8
	28.iii.1950	7	41,800	2,571		12.3
	30.v.1950	8	81,200	5,221		12.9
	25.vii.1950	8	42,600	8,959	42.0	

* Host restrained from licking for first 15 days only.

TABLE 1 (*Continued*)

Beast No.	Date of Infestation	Max. Age of Larvae (days)	No. of Larvae Applied	No. of Engorged Female Ticks Falling	Female Ticks Surviving (%)	
					Host Restrained from Licking	Host not Restrained from Licking
5	31.x.1949	13	30,300	1,368		9.0
	28.xi.1949	8	57,800	1,614		5.6
	23.i.1950	12	42,500	3,294		15.5
	7.iii.1950	8	55,600	313		1.1
	16.v.1950	7	46,800	3,117		13.3
	11.vii.1950	7	49,300	4,172		16.9
6	24.x.1949	6	33,000	471		2.9
	21.xi.1949	20	42,000	410		2.0
	18.iv.1950	7	42,400	12,428	58.6	
	13.vi.1950	7	56,000	3,059		10.9
	8.viii.1950	7	41,200	5,754	28.0	
7	17.x.1949	11	42,600	1,076	5.0*	
	14.xii.1949	13	29,000	95		0.6
	14.ii.1950	15	42,800	458		2.1
	4.iv.1950	12	60,200	801		2.7
	6.vi.1950	7	63,700	1,626		5.1
8	14.xi.1949	13	30,400	1,242		8.2
	16.i.1950	8	64,100	499		1.6
	7.iii.1950	8	68,100	25		0.1
	10.v.1950	20	22,200	4,107	36.9	
	18.vii.1950	7	53,100	7,579	28.6	
	5.ix.1950	7	42,600	3,260	15.3	
9	5.xi.1949	12	40,800	1,284		6.3
	16.i.1950	10	31,100	1,584		10.2
	7.iii.1950	12	82,500	1,987		4.8
	27.iv.1950	7	42,100	6,670		31.7
	27.vi.1950	7	66,900	7,368		22.0
	22.viii.1950	7	38,700	13,432	69.4	
10	20.xii.1949	7	34,900	276		1.6
	21.ii.1950	8	23,700	1,942		16.4
	11.iv.1950	9	46,400	4,415		19.0
	20.vi.1950	7	63,200	7,538		23.9
	15.viii.1950	9	70,300	10,408	29.6	

* Host restrained from licking for first 9 days only.

If a line with slope of 1.00 per cent. per month is fitted through the point of mean survival percentage and associated mean time for any animal, and the deviates of the survival percentages from the line are determined, then without exception the deviates for all trials with harness are positive and greater than the deviate for any trial without harness on the same animal.

The deviates for each of the nine animals which had trials with and without harness were ranked, and these ranks were converted to equivalent normal deviates (Fisher and Yates 1953), which were then averaged for the trials with and without harness. The analysis of the 2×9 non-orthogonal factorial arrangement indicates a highly significant effect ($P < 0.001$) of harness. The same conclusion is reached if, instead of using deviates from a fitted regression, the normal deviates equivalent to the ranks of the survival percentages, ignoring trend, are made the basis of the analysis.

IV. Discussion

The experiments demonstrated clearly that the yield from cultures was improved if the cattle were prevented from licking. The results have a wider significance, however, in that they suggest that licking should be taken into account in studies of the factors regulating tick abundance.

The extent to which the results indicate what happens under natural conditions cannot be stated with certainty. Licking is a normal toilet mechanism with cattle, even when they are devoid of ectoparasites, but appears to be intensified in stalled animals. It was repeatedly observed that painted identification marks disappeared far more rapidly from the coats of stalled cattle than from animals in pasture. However, since the cattle grazing naturally probably carried much smaller populations of larval ticks than the stalled cattle, further work is needed to assess the influence on survival of a smaller absolute amount of licking operating on lower populations of infesting larvae.

The stalled cattle appeared to display increased licking activity following infestation, particularly in the first few hours when large numbers of larvae were becoming attached. It was therefore expected that the amount of licking as reflected in the survival percentages would vary with the number of larvae originally applied, i.e. that licking would be density-reactive in the sense of Nicholson (1954), but this expectation was not fulfilled. However, such a reaction may have occurred, but been masked by the numbers of ticks developing on body areas unaffected by licking. Alternatively, the number of larvae applied may have been large enough to exceed the range in which a density-reactive licking response was elicited. The possibility must also be considered that licking may become excessive in stalled cattle, irrespective of tick infestation, in compensation for the loss of the normal pattern of daily activity. By their confinement the stalled cattle were prevented from taking part in various forms of normal behaviour, such as grazing, which normally occupies about 7 hours of the day (Larkin 1954), and play.

In spite of the high rate of licking by stalled cattle, some of the infestations on animals not impeded in licking produced considerable numbers of ticks, e.g. animals 4, 5, 9, and 10 dropped upwards of 3000 engorged female ticks from some of their infestations (Table 1). Although

there is considerable difference of opinion as to what constitutes a tick burden of economic significance, there is little question that the continuous fall of engorged females in such numbers from young beasts would have a serious effect on their well-being. Even exaggerated licking is clearly inadequate on its own as a natural mechanism for producing an economic degree of tick control, except perhaps on some animals. Other factors are known to influence the number of ticks on animals not receiving acaricidal treatments (Johnston and Bancroft 1918; Wilkinson 1955) to such an extent that some beasts seldom carry a tick burden of any consequence even on the parts of the body which the tongue cannot reach. The part played by the licking habit in determining the relative population density of ticks on various parts of the body of susceptible animals should, however, receive consideration.

Other forms of cattle behaviour which, like licking, produce tick mortality by mechanical means, e.g. rubbing or kicking, also warrant attention in the study of the natural mechanisms that regulate tick population.

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